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A New Material for the Control of the Pear Leaf Blister Mite[1]

O. H. Fullmer, E. A. Kurtz, and C. C. Cassil, *Niagara Chemical Division, Food Machinery and Chemical Corporation, Richmond, California*

Abstract

Phostex, bis(dialkyloxyphosphinothioyl) disulfides, where the alkyl groups are ethyl and 2-propyl in a ratio of three to one, is a phosphate insecticide-acaricide of low mammalian toxicity (acute oral LD_{50}, 2500 mg./kg. to white rats). Phostex has given excellent control of the pear leaf blister mite, *Eriophyes pyri* (Pgst.), on pears during 2 years of development and 1 year of commercial use. No injury was detected on pears when Phostex emulsifiable concentrate was used alone in the fall (postharvest) or delayed dormant or when Phostex was used in combination with oil during the dormant period. Phytotoxicity has been observed where Phostex was applied to the foliage of most stone fruits; however, no injury to these trees has been observed with dormant sprays of Phostex-oil combinations.

The pear leaf blister mite has been a major pest of pear trees for many years. Phostex, bis(dialkyloxyphosphinothioyl) disulfides, where the alkyl groups are ethyl and 2-propyl in a ratio of three to one, a new insecticide-acaricide of low mammalian toxicity (acute oral LD_{50}, 2500 mg./kg. to white rats), has provided excellent control of this mite during 2 years of development and 1 year of commercial use.

The damage caused by this mite occurs in the bud, on the leaf, and on the fruit. The damage to the bud is a result of mite feeding within the bud during the dormant period. Because floral parts may be damaged or destroyed, this feeding results in lighter bloom, as well as apple-shaped, russeted pears. The leaf injury (fig. 1) appears as round, raised spots on the upper leaf surface. These spots are red on young and tender foliage but darken as the leaf matures and finally appear as black dots. Lesions, similar to those occurring on the leaves, also occur on the fruit (fig. 2). They are initially reddish, then brown, and finally develop into russeted spots surrounded by a halo of clear tissue. The fruit is also often badly misshapen. These two factors make the blistered fruit unmarketable as fresh fruit.

It is believed by some workers, Stubbings (1947) and Borden (1932), that there are two different mite forms involved, one which has been designated as the bud mite and the other as the blister mite. The bud form is believed responsible for bud damage and bud failure but does not cause blistering. The other form is believed responsible for fruit and leaf blistering. Other workers, Essig (1924), Parker (1928), Newcomer & Rolfs (1932), and Kiefer (1952), have also made reference to the existence of a "bud strain." No morphological or biological differences between forms of these mites have ever been satisfactorily defined.

Fig. 1.—Bartlett pear shoot showing typical pear leaf blister mite injury (photograph courtesy H. F. Madsen, University of California at Berkeley).

[1] Accepted for publication January 8, 1959. Partial cost of publication of this paper was met by the Niagara Chemical Division, Food Machinery and Chemical Corporation, Richmond, Calif.

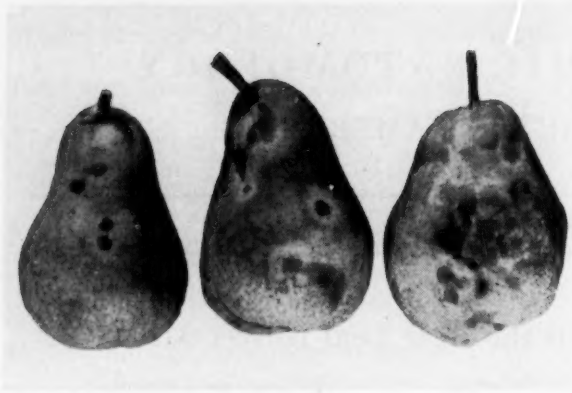

FIG. 2.—Bartlett pear fruit showing typical pear leaf blister mite injury (photograph courtesy H. F. Madsen, University of California at Berkeley).

Sulfur materials have long been used for the control of the pear leaf blister mite. These generally provide adequate control of blistering on leaves and fruit, but do not always prevent mite damage within the dormant buds. The mite kill within the buds with the sulfur materials is erratic and often very slow. This would seem to lend credence to the idea that two forms are present, assuming that the sulfur materials are effective on the blister form but not on the bud form.

Phostex has been shown to be extremely toxic to all mites within the buds and also very effective for the control of leaf and fruit blistering. If there are two forms, Phostex is apparently toxic to both.

Phostex thus offers a distinct advantage over the sulfur materials. By controlling the mites within the bud, the material prevents bud damage as well as leaf and fruit blistering.

The mode of action of Phostex on this mite is not known; however, observations of treated buds have shown dead mites as deep as the third and fourth bud scales.

PROCEDURE.—During the early experiments (1955–56), the plot work consisted of spraying known infestations of blister mites, and then examining the dissected fruit buds under a microscope to determine actual mite kill. In these trials, the buds examined were selected and tagged prior to leaf fall. The basis of bud selection was close proximity to badly blistered leaves. This assured blister mite infested buds. When this early work indicated excellent control of mites within the buds, the program was expanded into actual field trials using commercial equipment. Six of these trials were performed during the season of 1955–56 in mite-infested pear orchards. The size of each test plot was approximately 1 acre; plots were not replicated.

In 1956–57, experiments were again conducted under actual field conditions. Trials were established in all the major pear-growing areas in California and Oregon. Commercial applications of Phostex were made in 12 pear orchards during this season. The mite populations in these orchards at time of treatment ranged from very light to exceedingly heavy. In all cases, a two-spray Phostex program (postharvest followed by dormant or delayed dormant) was compared with a single postharvest spray. The size of each unreplicated plot was again approximately 1 acre. All trials were compared directly with an accepted program which varied as to materials and number of sprays according to grower preference. In many instances completely unsprayed checks were also available for comparison.

Evaluation of all trials (1955–57) was made by visual observation on the number of infested fruit and leaf clusters per tree. Usually 15 to 20 trees were examined at random in each plot area. In some trials actual mite kill within buds was also determined.

Chemicals employed in all trials have been designated in the tables as concentrates (C) or as toxicants dissolved in dormant oil[2] (TO). The numbers associated with these formulation designations refer to the pounds of active ingredient per gallon.

RESULTS AND DISCUSSION.—The results of the 1955–56 experiments were exceedingly promising. Mite kill within the buds was excellent in all cases. In three of the trials the control of leaf- and fruit-blistering with Phostex was equal to that obtained with the standard materials. In the other three experiments the standard applications were slightly superior to the Phostex treatments although both standard and Phostex sprays gave excellent control of blistered fruit and leaves when compared with untreated trees.

The control of blister mite with Phostex during the 1956–57 season was excellent. A single postharvest spray provided practically complete control in 8 of the 12 orchards and excellent control in the remaining 4 orchards. In the two-spray program, virtually complete control was obtained in all 12 orchards. In comparison with the Phostex control, the standard programs provided essentially complete control in 10 of the 12 orchards.

The data given in the tables are typical of the experimental results obtained over the 2-year period. The data in table 1 compares the effectiveness of Phostex and lime-sulfur for the control of mites within the dormant buds. The mite kill within the buds sprayed with Phostex was excellent, while the lime-sulfur failed to give any reduction over the check. Tables 2 and 3 represent typical examples of control with Phostex where the evaluation was made by visual observations of blistered fruit and leaves.

The data in table 2 represent blister mite control under high population conditions. During the previous season, one-third of the fruit in this orchard was damaged by blister mite. In this test a single Phostex spray (post-

[2] Viscosity—SSU 120 at 100° F., UR—80.

Table 1.—Results of treatments against blister mite on Bartlett pears. Newcastle, California, 1955–56.

MATERIAL POSTHARVEST SEPTEMBER 8, 1955	DOSAGE PER 100 GALS.	NUMBER OF LIVE BLISTER MITES PER SELECTED BUDS AFTER[a]		
		27 Days	76 Days	125 Days
Phostex 8.0 (C)	1 pt.	0.0	0.1	0.2
Lime-sulfur	5 gals.	—	44.6	75.0
Untreated	—	107.3	31.8	80.8

[a] All counts made on basis of 10 buds per treatment.

Table 2.—Results of treatments against blister mite on Bartlett pears. Upper Lake, California, 1956–57.

MATERIAL POSTHARVEST SEPT. 19, 1956	DOSAGE PER 100 GALS.	MATERIAL DELAYED DORMANT MAR. 10, 1957	DOSAGE PER 100 GALS.	NO. OF BLISTERED FRUIT AND LEAF CLUSTERS/15 TREES ON MAY 9, 1957
Phostex 8.0 (C)	1 pt.	Phostex 8.0 (C)	1 pt.	0
Phostex 8.0 (C)	1 pt.	—	—	2
Lime-sulfur Kolospray[a]	3 gal. 5 lbs.	Lime-sulfur Kolospray[a]	3 gal. 5 lbs.	2
Untreated	—	—	—	750

[a] A fused bentonite sulfur product containing 81% sulfur.

Table 3.—Results of treatments against blister mite on Bartlett pears. Auburn, California, 1956–57.

MATERIAL POSTHARVEST OCT. 9, 1956	DOSAGE PER 100 GALS.	MATERIAL DORMANT JAN. 17, 1957	DOSAGE PER 100 GALS.	NO. OF BLISTERED FRUIT AND LEAF CLUSTERS/15 TREES ON MAY 1, 1957
Phostex 8.0 (C)	1 pt.	Phostex .76 (TO)	1 gal.	4
Phostex 8.0 (C)	1 pt.	Dormant Flow. Emulsion Oil	4 gal.	4
Lime-sulfur	5 gal.	Lime-sulfur Dormant Flow. Emulsion Oil	5 gal. 4 gal.	119
Untreated	—	—	—	734

harvest) was compared with two applications of Phostex (postharvest and delayed dormant). These were compared with a standard two-spray program of lime-sulfur and Kolospray[3] (postharvest and delayed dormant). The materials were applied by air-carrier equipment at 700 gallons per acre in the postharvest spray and 400 gallons per acre during the delayed dormant period. All spray programs provided excellent control of blistering while the unsprayed check trees were badly blistered.

Table 3 summarizes the work done in an orchard that had only a low population of mites during the previous year. The comparison of a single Phostex application (postharvest) against two sprays (postharvest and dormant) was made along with the grower's application of lime-sulfur (postharvest) followed by a dormant spray of lime-sulfur and dormant oil. The materials in this trial were applied by air-carrier equipment at 500 gallons per acre for the postharvest application and 350 gallons per acre during the dormant period. The results as shown in the table clearly indicate the excellent control obtained with either one or two applications of Phostex. The lime-sulfur gave considerable control over the untreated area but was not equivalent to the control obtained with Phostex.

In addition to controlling blister (bud) mites, Phostex has also shown toxicity to other pests. In the western pear-growing areas, the postharvest application of Phostex emulsifiable concentrate has aided in the control of the European red mite, *Panonychus ulmi* (Koch), the two-spotted spider mite, *Tetranychus telarius* (L.), and the pear psylla, *Psylla pyricola* Foerst. During the dormant season, Phostex and dormant oil combination sprays have provided excellent control of San Jose scale, *Aspidiotus perniciosus* Comst., European fruit lecanium, *Lecanium corni* Bouché, soft scale, *Coccus hesperidum* (L.), and the frosted scale, *L. pruinosum* Coq. Applications of Phostex and dormant oil during the delayed dormant period have given excellent control of the eggs of the rosy apple aphid, *Anuraphis roseus* Baker; hop aphid, *Phorodon humuli* (Schr.); and the mealy plum aphid, *Hyalopterus pruni* (Geoffroy).

Injury to pear trees has not been observed with Phostex when applied either alone as an emulsifiable concentrate in the fall or when added to oil in the dormant or delayed dormant period. Foliage injury has occurred to most stone fruits with summer applications of Phostex but no injury has been observed when the same varieties have been treated during the dormant period with Phostex and oil combinations.

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[3] A fused bentonite sulfur product containing 81% sulfur.

NOTICE TO AUTHORS

Each manuscript submitted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY must be accompanied by an abstract suitable for publication, and prepared in accordance with instructions for the preparation of abstracts as required by *Biological Abstracts*. This abstract will replace the usual author's summary. See "Important Notice to Authors" which appeared in the *Bulletin of the Entomological Society of America* 3(2): 1. June, 1957.

Notes on Phytophagous and Predatory Mites of Arizona[1]

F. F. Bibby, *Arizona Fertilizers, Inc., Phoenix*
and
D. M. Tuttle,[2] *University of Arizona Agricultural Experiment Station, Yuma*

Abstract

This paper is an annotated list of 38 Arizona species of phytophagous mites, 2 of which are probably undescribed, and of 13 species of predatory mites, 3 of which are probably undescribed. The list is based upon collections made by the writers and associates during the past 6 years and upon collections of others in the meantime and previously. The host(s), place(s) and date(s) of collection, an occasional notation as to degree of infestation and other information, considered pertinent and timely, are included.

Of the phytophagous mites, the records indicate that *Tetranychus cinnabarinus* (Bois.) is the dominant species, with *T. desertorum* Banks, *T. telarius* (L.), *Petrobia latens* (Müll.) and *Bryobia praetiosa* Koch following in the order given. Of economic importance to crops in Arizona, the data contained in the paper indicate that the above named species rank in the same order as their dominance, with *Oligonychus pratensis* (Banks) probably coming next, in either category.

In the annotated list of mites given below, there are 38 phytophagous species (2 probably undescribed) and 13 predatory species (3 probably undescribed) collected in Arizona during the past 6 years by the writers and associates and by other collectors in the meantime and previously. General references to the economic species are given under "References Cited" at the end of this paper.

Of the phytophagous mites, the records indicate that *Tetranychus cinnabarinus* (Bois.) was probably the most prevalent species, with *T. desertorum* Banks, *T. telarius* (L.), *Petrobia latens* (Müll.) and *Bryobia praetiosa* Koch following in the order given. As a pest of cotton and cantaloupe, *Tetranychus cinnabarinus* (Bois.) seemed to be the worst. On grain sorghum, *Oligonychus pratensis* (Banks) dominated, with *O. stickneyi* (McG.) next. Three species were abundant on alfalfa: namely, *Tetranychus cinnabarinus* (Bois.), *T. telarius* (L.) and *Bryobia praetiosa* Koch. *Petrobia latens* (Müll.) usually dominated on onions and occasionally on other truck and garden crops and on alfalfa.

Phytophagous Mites:

Aceria fixus (Cotte), fig. mite (Eriophyidae)

Fig.: Tucson (Reid Ranch) X-10-46.

Aceria sp. (prob. undescribed) (Eriophyidae)

Franseria ambrosiodes: Black Canyon Highway (Maricopa Co.), X-20-57, possibly causing leaf galls on host.

Brevipalpus aeoloides Pritchard and Baker (Tenuipalpidae)

Encelia farinosa: Glendale, X-18-53.
Franseria deltoidea: Papago Park, Tempe, VIII-9-53.

Brevipalpus californicus (Banks) (Tenuipalpidae)

Pittosporum: Mesa, VIII-6-54, severe.

Brevipalpus cardinalis (Banks) (Tenuipalpidae)

Ash: Phoenix, III-7-51 (M. H. Frost).
Cucumber: Phoenix, I-24-1927 (Comm. Agric. Hort.) (?)

Brevipalpus lewisi McGregor (Tenuipalpidae)

Citrus: Tempe, X-9-53 (L. Hopkins), common.

Bryobia drummondi (Ewing) (Tetranychidae)

Larrea tridentata (creosotebush): Picacho, October 1953 (J. Warren).
Prosopsis juliflora (mesquite): Toltec (Pinal Co.), IX-21-53 (J. Warren).

Bryobia praetiosa Koch, clover mite (Tetranychidae)

Alfalfa: Buckeye, III-22-43 and Chandler, IV-6-49 (E. E Russell); Phoenix, April, 1929; Yuma, II-10-54 (D. C McCain), III-31-48 (M. H. Frost), II-15-, II-20- and II-24-52, II-2-, III-2-, III-13- and III-21-53.
Almond: Scottsdale, 1923 (Comm. Agric. Hort.), causing the leaves of several trees to shed.
Apple: Willcox, VI-1-49 and Holbrook, VIII-30-50 (L. P. Wehrle) and Sedona, V-23-44 (J. N. Roney).
Bachelor button: Tucson, I-31-54 (G. D. Butler, Jr.).
Bur clover: Tucson, IV-13-54 (G. D. Butler, Jr.).
Calendula: Tucson, IV-4-54 (G. D. Butler, Jr.).
Clover: Flagstaff, IV-24-38 and XII-24-38 (L. P. Wehrle) and Tucson, III-20- and 21-52 (G. D. Butler, Jr.).
Cucumber: Tucson, IV-6-45 (L. P. Wehrle).
Dwarf marigold: Phoenix, II-28-54 (M. F. Wharton, Jr.).
Malva sp.: Arrowhead Ranch (Maricopa Co.), III-26-48 (M. H. Frost).
Morning-glory (Convolvulaceae): Tucson, IV-4-33 (L. P. Wehrle).
Mustard: Tucson, IV-1-39 (R. B. Streets).
Pear: Warren, VII-2-45 and Safford, IX-13-50 (L. P. Wehrle).
Radish: Tucson, IV-6-45 (L. P. Wehrle), causing leaves to appear grayish.
Rye: Phoenix, IV-3-38 (K. B. McKinney), and on ornamentals and weeds, and indoors.

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[2] Authors' names listed in alphabetical order, neither seniority nor precedence being implied.

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Rye grass: Tucson II-19-52 (G. D. Butler, Jr.), abundant in lawn.
Squash: Tucson, IV-6-45 (L. P. Wehrle); Yuma, I-10-54, causing noticeable damage to leaves.

Eotetranychus frosti (McGregor) (Tetranychidae)

Rose: Tempe (type locality, McGregor 1952) V-12-49 (M. H. Frost), specimens from which species was described, not reported on any other host in Arizona, but recorded from raspberry in California by Pritchard and Baker (1955).

Eotetranychus malvastris (McGregor) (Tetranychidae)

Cassia sp.: Tucson, XI-26-1953 (L. A. Carruth).
Sphaeralcea sp.: Luke Field (Maricopa Co.), III-10-57 and V-12-57, and from other mallows in Arizona and California, according to Pritchard and Baker (1955).

Eotetranychus sexmaculatus (Riley), six-spotted mite (Tetranychidae)

Citrus: Yuma, 1945 federal survey crew.

Eotetranychus talisiae (Hirst) (Tetranychidae)

Sphaeralcea sp.: El Mirage, I-31-51.

Eotetranychus weldoni (Ewing) (Tetranychidae)

Cottonwood (*Populus* sp.): Yuma, X-15-53 (Czajkowski & McCain).

Eotetranychus yumensis (McGregor) (Tetranychidae)

Castorbean: Yuma (type locality, McGregor 1934), XI-6-52, very numerous on underside of leaves.
Citrus: Yuma, IV-4-53, in scattered localities on orange and grapefruit leaves, and according to Pritchard and Baker (1955), abundant on citrus in Coachella and Imperial Valleys of California.
Grain sorghum: Yuma, XI-10-53 (D. C. McCain).

Eriophyes pyri (Pgst.), pear leaf blister mite (Eriophyidae)

Pear: Bisbee, June 1945, and Phoenix, 1926 (Comm. Agric. Hort.) and VI-3-49 (L. P. Wehrle).

Heterotergum sp. (probably new) (Eriophyidae)

Franseria ambrosiodes: Black Canyon Highway (Maricopa Co.), X-20-57, in association with other mites.

Histiostoma sp. (Anoetidae)

Corn: Gilbert, VIII-18-56, hypopi, small traveling stage of mite, with larval stage of *Zeadiatrea grandiosella* (Dyar) within tunnel.
Orange: Mesa, X-9-36, hypopi stage, on navel end rot in Val-Vista Orchard.

Hystrichonychus gracilipes (Banks) (Tetranychidae)

Sida heracea: Yuma, IV-18-55, a new host record according to Pritchard and Baker (1955).
Sphaeralcea sp.: Luke Field (Maricopa Co.), III-10- and V-12-57, and El Mirage, I-31-54.

Neotetranychus siccus Pritchard & Baker (Tetranychidae)

Olneya Tesota: Papago Park, Phoenix, IX-27-53, specimens from which the species was described by Pritchard and Baker (1955), without the name of the host being included.

Oligonychus coniferarum (McGregor) (Tetranychidae)

Italian cypress: Mesa, III-25-55 (L. Hopkins) and IV-3-55.

Oligonychus modestus (Banks) (Tetranychidae)

Corn: Navajo and Apache Counties (Comm. Agric. Hort.).

Oligonychus platani (McGregor) (Tetranychidae)

Pyracantha: Tucson, V-9-52 (Willsey) and March 1954 (G. D. Butler, Jr.)

Oligonychus pratensis (Banks) (Tetranychidae)

Bermuda grass: Tolleson, Sept. 1954, and Yuma, X-15-53 (D. C. McCain).
Corn: Safford, IX-5-52 (R. B. Streets).
Date: Yuma, 1945 (Comm. Agric. Hort.), of economic status.
Grain sorghum: Gilbert, IX-2-53 (I. McMahon); Peoria, X-13-53; Phoenix, X-25-53; Roll, VI-25-55 (H. Brown); Scottsdale, IX-1-53 (J. Hartsig) and Yuma, X-29-53 (H. Czajkowski).

Oligonychus stickneyi (McGregor) (Tetranychidae)

Bermuda grass: Yuma, X-15-53 (D. C. McCain)
Corn: Maricopa, V-16-56 (H. Brown) and Tempe, V-18-56 (J. Hartsig).
Grain sorghum: Litchfield Park, V-29-56; Phoenix, X-12-56, and Sunnyslope, IX-21-52 (L. Hopkins).

Oligonychus ununguis (Jacobi) (Tetranychidae)

Arborvitae: Phoenix, IV-2-27 (C.D.L.); Tucson, XII-15-50 (R. Simpson).
Italian cypress: Tucson, IV-15-, V-15- and XII-2-53 (G. D. Butler, Jr.).

Pentamerismus sp. nr. *baptus* Pritchard & Baker (Tenuipalpidae)

Franseria deltoidea: Papago Park, Tempe, 1-29-54.

Petrobia latens (Müller), brown wheat mite (Tetranychidae)

Alfalfa: Beardsley, II-23-55 and Buckeye, III-23-55 (B. Zimmer); Casa Grande, II-27-56 (R. L. Dezember); Chandler, IV-6-49 (E. E. Russell); Perryville, IV-13-55; Pinal Co., IV-8-55, and Yuma, II-10-54 (D. C. McCain).
Barley: Luke Field (Maricopa Co.), IV-5-55 (L. A. Hammers).
Bermuda grass: Roll, V-12-54 (L. L. Brimhall).
Cantaloupe: Luke Field (Maricopa Co.), IV-5-55 (L. A. Hammers) and Tolleson, V-25-48 (M. H. Frost).
Carrot: Deer Valley, IV-14-48 (M. H. Frost).
Cotton: Scottsdale, May 1954, and Yuma, IV-19-55 (D. C. McCain).
Endive: Yuma, III-15-52, small acreage unmarketable due to damage by this mite.
Lettuce: Alhambra, IV-7-49 (M. H. Frost).
Lupinus sp.: Tom Mix Monument (Pinal Co.), III-26-55.
Onion: Deer Valley (Maricopa Co.), IV-20-51 (M. H.

Frost); Phoenix, IV-12-54 and III-21-55 (W. R. Ritchie); of economic status in all instances.

Rhizoglyphus echinopus (F. & R.) (Acaridae)

Cotton: Phoenix, V-14-55 (S. Evans), on rotting seed, and VI-8-55 (B. Fehrman), at base, above ground, on recently emerged plant.

Scheloribates sp. (Oribatulidae)

Johnson grass: Phoenix, I-24-54, from frost bitten material.

Tetranychus atlanticus (McGregor), strawberry spider mite (Tetranychidae)

Alfalfa: Yuma, X-20-52 and VI-14-55.
Castorbean: Gilbert, IX-20-56 (P. D. Gerhardt).
Cotton: Buckeye, VI-13-56 and Marinette, V-31-56 (B. Zimmer); Yuma, VI-3- and 23-53 and IV-19-55.
Soybean: Coolidge, IX-28-54 and Goodyear, IX-22-55, severe.

Tetranychus canadensis (McGregor) (Tetranychidae)

Chinaberry: Tempe, IX-17-52 (L. Hopkins).

Tetranychus cinnabarinus (Boisduval) (Tetranychidae)

Alfalfa: Yuma, VII-12-51 (G. D. Butler, Jr.).
Cantaloupe: Luke Field (Maricopa Co.), IV-18-58 (D. Berry) and Phoenix, VI-10-53 (O. A. Hills).
Castorbean: Chandler, IX-20-56 (P. D. Gerhardt).
Corn: Gilbert, VII-6-56, and Yuma, VI-20-55 (D. C. McCain), severe.
Cotton: Buckeye, VII-27-56 (C. Davis); Chandler, IX-20-56 (P. D. Gerhardt); Dateland, V-9-57 (P. Segulja), on sucker cotton, severe and persistent; Eloy, V-7-56 and VIII-14-57 (Hornney & Pace); Gilbert, VIII-6-56 (Frost & McMahon); IX-20-56 (P. D. Gerhardt); Glendale, IX-3-56 (F. Corbus); Litchfield Park, VII-14- and VIII-3-55; Luke Field (Maricopa Co.), VII-18-55, VI-14-56 (B. Zimmer) and X-16-58; Maricopa, V-16-56 (H. Brown); Marinette, IV-25-26 (B. Zimmer); Mesa, VII-10-57 (R. L. Dezember); Peoria, VI-14-56; Phoenix, VI-14-55, VII-28-55 (B. Zimmer), V-10-56 (W. R. Ritchie), V-19-56 (B. Zimmer) and IX-5-56 (F. Corbus); Tolleson, VIII-3-58 and Yuma, VI-20-55.
Cauliflower: Peoria, XI-24-53 (L. A. Hammers).
Dwarf marigold: Phoenix, VI-29-58, severe with extensive webbing.
Hollyhock: Casa Grande, V-1-56 (K. Tipling), severe.
Lettuce: Maricopa Co., IX-26-55, light infestation.
Malva parviflora: Phoenix, V-14-57, heavily infested, and Tempe, IX-18-56 (J. Hartsig).
Morning-glory (Convolvulaceae): Phoenix, IX-13-57 (D. Berry).
Rose: El Mirage, VI-3-58 (C. Ash), severe, and Phoenix, V-5-56, severe.
Sapindus sp. (soapberry): Phoenix, VI-23-57.
Soybean: Maricopa Co., IX-26-55.
Sugar beet: Chandler, V-5-58 (R. L. Dezember); Litchfield Park, V-25- and 27-57 (B. Fehrman), I-31-58 (S. Evans), being devoured by larvae and adults of *Stethorus atomus* Csy. (det. W. H. Anderson), and V-29-58 (D. Berry) and Phoenix, V-30-55 (B. Zimmer) and V-24-56 (F. Corbus).
Tomato: Litchfield Park, VI-10-56 (G. Wene), severe.
Tribulus terrestris: Phoenix, XI-28-58.
Watermelon: Eloy, VI-10-55 (J. Warren); Phoenix, VI-26-56, severe and heavily webbed, and Yuma, V-1-55.

Tetranychus desertorum (Banks) Tetranychidae)

Alfalfa: Tempe, V-10-48 (M. H. Frost).
Cantaloupe: Deer Valley (Maricopa Co.), IV-3-51; Luke Field (Maricopa Co.) IV-5-55 (L. A. Hammers); Phoenix, IV-20-48 (M. H. Frost), June 1951 (E. A. Taylor), VI-10-53 (O. A. Hills), IV-18-53 (Taylor & Sheets), VI-2-54 (L. A. Hammers) and IV-5-55; Tolleson, V-25-48 (M. H. Frost) and Yuma, III-5-52 and VI-2-53, IV-11-55 (A. Bell) and V-2-55.
Celery: Luke Field (Maricopa Co.), IX-2-49, and Phoenix X-18-45 (R. B. Streets).
Corn: Eloy, V-29-56 (K. Brinkmeyer) and Maricopa V-16-56 (H. Brown).
Cotton: Avondale, VI-15-53 (L. Sheets); Avra Valley (Pima Co.), VII-10-53 (H. Karel); Buckeye, IX-1-50 (L. Sheets); Casa Grande, VII-3-56 (R. L. Dezember); Continental, V-24-50 (Stevenson & Kauffman), VI-20-51 (Kauffman & Sheets) land VI-25-51 (Kauffman); Deer Valley (Maricopa Co.), VII-12-50 (L. Sheets) and VI-29-51 (Stevenson & Sheets); Elfrida, X-2-53 (G. D. Butler, Jr.) and X-5-53 (C. Allen); Eloy, VII-5-51 (Kauffman & Sheets); Gilbert, IX-2-53 (I. McMahon); Higley VI-12-53 (L. Sheets); Litchfield Park, VII-14-55; Luke Field, VII-18-55; Marana, VIII-10-48 (Wm. Kauffman) and VIII-30-49 (L. Nickelson); Maricopa, IX-5-56 (H. Brown); Marinette, VII-1-48 (M. H. Frost), VI-16-53 (L. Sheets) and V-31-56 (B. Zimmer); Mesa, IV-23-51 and VI-29-53 and Palo Verde, IX-12-50 (L. Sheets); Phoenix, VI-14-, VI-28- and VIII-3-55; Queen Creek, VI-13-56 (I. McMahon); Safford, VIII-29-49 (M. H. Frost), VI-30-50 (Wm. Kauffman) and VI-22-51 (Kauffman & Sheets); Sahuarita, VIII-8-53 (H. Karel); Stanfield, IX-9-56 (H. Brown); Toltec, VIII-25-53 (J. Warren) and Yuma, VI-20-55.
Cressa truxillensis (Convolvulaceae); Yuma, V-2-55, severe and spreading to cantaloupe fields.
Larrea tridentata: Picacho, Oct. 1953 (J. Warren).
Lettuce: Phoenix, III-21-55.
Lupinus sp.: Tom Mix Monument (Pinal Co.), III-26-55.
Malva parviflora: Somerton, IV-7-55.
Sorghum: Tucson, II-10-43 (R. B. Streets).
Sphaeralcea sp.: Deer Valley, IV-3-51 (M. H. Frost).
Strawberry: Phoenix, I-25-50 (Hills & Taylor).
Sugar beet: Phoenix, III-15-51 (E. A. Taylor) and V-20-53 (O. A. Hills).
Sweet Pea: Maricopa Co., Dec. 1953.
Tribulus terrestris: Luke Field, X-20-53 (L. A. Hammers); Phoenix, XI-28-58, severe, being attacked by *Scolothrips sexmaculatus* (Perg.) (det. K. O'Neill).
Watermelon: Coyote Mts. (Pima Co.), VII-13-42 (R. J. Moody) and Mesa, VI-4-55.
Zinnia: Casa Grande, X-28-53 (J. Warren).

Tetranychus mcdanieli (McGregor) (Tetranychidae)

Leguminous weed: Christopher Creek (Gila Co.), VII-20-58 (C. R. Ash).
Mulberry: Tucson, IV-14-54 (G. D. Butler, Jr.).

Tetranychus telarius (Linnaeus), two-spotted spider mite

Alfalfa: Mesa, VIII-13-51 and Somerton, VII-2-51 (G. D. Butler, Jr.); Tempe, V-18-56 (J. Hartsig) and Yuma, VI-8-50, VI-9-50 (R. Simpson), VI-18-51 (L. A. Carruth), VII-4-, 10- and 12-51 (G. D. Butler, Jr.) and III-21-53.

Aralia sp.: Tucson, III-20-52.

Banana: Phoenix, V-23-53 (Brantlinger), infesting trees in nursery.

Cantaloupe: Deer Valley, V-24-53 (L. A. Carruth); Phoenix, VII-11-51 (E. A. Taylor) and VI-2- and 17-54 (L. A. Hammers), Pinal Co., V-5-54 (J. Warren); Yuma, IV-18-53 (G. D. Butler, Jr.) and V-27- and 28-53.

Castorbean: Tolleson, VII-7-54.

Celery: Phoenix, X-7-53 (W. R. Ritchie).

Citrus: Mesa, III-25-55 (L. Hopkins).

Cotton: Litchfield Park, VII-14-54 (B. Zimmer), economic infestation; Peoria, VII-8-48 (Sheets & Breazeale) and VI-21-48 (L. Sheets); Phoenix, IX-14-53 (B. Fehrman), VI-28-54 (S. Evans) and VII-20-54 (L. A. Hammers); Safford, VIII-19-49, VI-30- and IX-8-50 and Sept. 1952 (Wm. Kauffman); Scottsdale, VII-26-52 (M. H. Frost), IV-25-53 (J. N. Roney) and IX-1-53 (J. Hartsig); Sunnyslope, IX-21-52 (L. Hopkins); Thatcher, VI-22-51 (Kauffman & Sheets); Tucson, V-17-48 (M. H. Frost); Waddell, VI-16-53 (L. Sheets) and Yuma, VII-5-51 (Kauffman & Sheets), IV-14- and 21-53, XII-1-53 (H. Czajkowski) and V-25-54.

Cottonwood (*Populus* sp.): Eloy, VII-5-54 (J. Warren).

Honeysuckle: Phoenix, V-16-53 (Brantlinger).

Ivy: Phoenix, II-17-55 (F. Schiller).

Lettuce: Deer Valley, XI-10-52 (L. Hopkins); Phoenix, X-23-53 (L. A. Hammers) and III-21-55 (S. Evans) and Yuma, VI-3-53, XI-30-53 (H. Czajkowski) and IV-7-55 (McCain & Evans).

Pear: Safford, IX-13-50 (L. P. Wehrle).

Philodendron: Tucson, III-30-52 (G. D. Butler, Jr.).

Pyracantha: Tucson, VI-1-44 (L. P. Wehrle).

Romaine lettuce: Yuma, III-7-53.

Soybean: Goodyear, IX-23-55, of economic status, and Phoenix, X-9-55.

Strawberry: Deer Valley, I-22- and III-14-52 (E. A. Taylor).

Sugar beet: Phoenix, IV-25-51 (E. A. Taylor) and V-15-53 (O. A. Hills).

Tobacco, wild: Phoenix, VII-20-51 (M. H. Frost).

Violet: Phoenix, XII-30-53 (B. Fehrman).

Watermelon: Pinal Co., V-15-54 (J. Warren); Tolleson, X-23-53 (B. Zimmer) and Yuma, VII-12-53 and V-25-54.

Tetranychus tumidus (Banks) (Tetranychidae)

Cotton: Safford, IX-1-50 (Wm. Kauffman).

Tyrophagus sp. (Acaridae)

Cantaloupe: Deer Valley (Maricopa Co.), III-27-47 (M. H. Frost).

Cotton: Buckeye, XI-7-57 (L. W. Sheets).

Vasates lycopersici (Massee), tomato russet mite (Eriophyidae)

Tomato: Tucson, VII-14-47 (L. P. Wehrle) and Yuma, IX-27-54, plants dying.

PREDATORY MITES:

Amblyseiopsis n. sp. (Phytoseiidae)

Bermuda grass: Roll, V-27-50, an enemy of the tetranychid mites.

Balaustium aonidaphagus (Ebeling) (Erythraeidae)

Alfalfa: Yuma, IV-26-52.

Balaustium sp. (Erythraeidae)

Alfalfa: Buckeye, IV-28-49 (E. E. Russell).

Cantaloupe: Yuma, IV-19-53.

Franseria ambrosiodes: Black Canyon Highway (Maricopa Co.), X-20-57, a larva along with other mites.

Hollyhock: Phoenix, IV-23-55.

Lycium sp.: Mesa, IV-3-55; Pinal Co., III-4-54 (J. Warren).

Mustard: Litchfield Park, III-25-55 and Salome, III-10-57.

Phacelia sp.: Peralta Canyon (Pinal Co.), III-31-57.

Sphaeralcea sp.: Tempe, III-30-57.

Cheyletia sp. (Cheyletidae)

Bermuda grass: Yuma, X-15-53 (D. C. McCain), probably a free-living predator.

Erythraeous sp. (Erythraeidae)

Alfalfa: Buckeye, IV-11-43 (E. E. Russell).

Garmania n. sp. (Aceosejidae)

Alfalfa: Queen Creek, III-26-51 (M. H. Frost), on roots, probably a predatory species.

Hemisarcoptes malus (Shimer) (Hemisarcoptidae)

Jasminum revolutum: Tucson, XII-22-36 (L. P. Wehrle), under *Parlatoria oleae* (Colvée) on leaves of the *Jasminum*.

Leptus sp. (Erythraeidae)

Alfalfa: Yuma, IV-20-52 (G. D. Butler, Jr.), larvae predaceous on insects.

Parasitus sp. (Parasitidae)

Sidewalk: Tucson, III-20-54 (G. D. Butler, Jr.)

Pronematus californicus (McGregor) (Tydeidae)

Citrus: Mesa, VIII-28-53 (L. Hopkins), a predator of citrus bud mite, *Aceria sheldoni* (Ewing).

Raphignathus sp. undescribed (Raphignathidae)

Barley: Roll, IX-22-54 (D. Cox), abundant on surface of stored barley and walls of storage shed, and Tucson, X-4-54 (J. R. Carter), abundant in stored grain.

Tydeus sp. (Tydeidae)

Alfalfa: Roll, III-15-57 (P. Segulja) and Yuma, VI-14-56.

Bermuda grass: Yuma, V-20-55 (D. C. McCain).
This mite may be predatory on other mites and their eggs.

Typhlodromus sp. (Phytoseiidae)

Bermuda grass: Yuma, V-20-55 (D. C. McCain).
Corn: Gilbert, VIII-18-56, with larval stage of *Zeadiatrea grandiosella* (Dyar) within tunnel, and Yuma, VI-20-55 (D. McCain).
Cotton: Buckeye, XI-7-57 (L. W. Sheets), in association with another mite.

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Rapid Desiccation of Drywood Termites with Inert Sorptive Dusts and Other Substances[1]

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ABSTRACT

Previous efforts to control insects by means of desiccating dusts have been directed primarily against granary weevils and with abrasive materials. Drywood termites were found to be more susceptible to sorptive than to abrasive dusts. By means of a staining technique it was shown that sorptive dusts can remove the lipoid protective layer covering the epicuticle, causing the termites to lose water rapidly. The insects needed only to crawl about on a thin film of the dust. They died after losing about 30% of their body weight in water. Certain silica aerogels were particularly effective. Other insects, including cockroaches, fleas, bedbugs, bees, ants, vinegar flies, mosquitoes, house flies, thrips, ticks and mites, likewise succumbed rapidly to desiccation initiated by the disruption or removal of the lipoid protective layer by sorptive dusts. Although some conventional insecticides might cause more rapid knockdown, they never resulted in death of the insects tested as rapidly as the more effective desiccating dusts.

Certain water-soluble fluorides have been incorporated into the silica aerogels in the process of manufacture. These fluorides greatly increase the effectiveness of the impregnated aerogels, even at low relative humidities, but the advantage obtained from the impregnation increases with increasing relative humidities and consequently increases the versatility of the silicas.

The presence of fluorides as monomolecular layers in the porous silica aerogel particles does not decrease the ability of these particles to adsorb wax. After the wax is adsorbed or disrupted, the water-soluble fluorides can act as contact insecticides. In addition, the strong positive charge they impart to the particles greatly increases their ability to adhere to the dusted surfaces.

The addition of surface-active solutes to petroleum oil will cause increased rate of water loss, the water appearing in the form of many small droplets. Desiccation is greatly enhanced when the applied oil is later removed and the insect cuticle is thereby directly exposed to air. Death may result more rapidly than by the toxicity or suffocation that takes place when the insects are completely immersed in oil.

Desiccation of drywood termites was caused by treatment with parathion, sodium fluoride, and anhydrous magnesium perchlorate. When the treated insects, as well as those shaken in a sorptive dust, were immersed in oil, water droplets appeared on the body surfaces.

In southern California the incidence of drywood termites, *Kalotermes minor* (Hagen), is about as great as that of the subterranean termite, *Reticulitermes hesperus* (Banks). Control of drywood termites, particularly when it involves fumigation of the infested dwelling with methyl bromide, is an expensive treatment. The fumigation, while highly effective against the existing infestations, leaves no residual protection against subsequent attacks. If it were possible to apply an effective, long-lasting residual insecticide to the rough frame of a house during or immediately following construction, or to the inside of the attic and other vulnerable areas after a fumigation or other type of treatment, this would be an important advance in the control of drywood termites. The residue should be effective for 5 to 10 years, or, ideally, for the life of the dwelling.

Ebeling & Pence (1956) applied dusts, mists and vapors of lindane, chlordane, dieldrin, DDT and toxaphene to blocks of wood and drywood termites were allowed to crawl over them. The relative effectiveness of the insecticides, and of the three formulations, was recorded. Dust was found to be the most effective formulation per unit of insecticide applied, in contrast to the usual experience in pest control.

The blocks of treated wood were placed in an attic for 17 months. Drywood termites were again placed on these blocks in July, 1957. By that time only the dusts were effective, indicating that the toxicants known to be effective initially in all formulations had lost their effectiveness, during the 17 months that the blocks had remained in the attic, except when formulated as dusts. Thus the only formulation remaining effective was the one leaving a residue of chemically inert diluents.

Inert diluents were then lightly dusted onto new blocks

[1] Accepted for publication July 21, 1958.

of wood and some were found to be strikingly effective in killing the drywood termites placed on them. The termites were killed by rapid desiccation (fig. 1). At a temperature of 75° F. and a relative humidity of 80%, alate termites were killed by the more effective common dust diluents (highly sorptive clays) in 7 to 9 hours and by finely divided powders of silica aerogels and silica precipitates available at that time in 5 to 7 hours. Regardless of the period required to kill them, however, the termites on the treated blocks never fed on the wood, whereas on untreated wood they began to feed after crawling about for 2 or 3 hours. The more effective diluents killed the termites much more rapidly than they were killed by the insecticide dusts used by Ebeling & Pence (1956), for the latter were prepared with less lethal diluents, such as pyrophyllite and talc.

The present paper has for one of its purposes an appraisal of some of the physical and biological factors that appear to influence the effectiveness, as desiccating agents, of the inert sorptive dusts as well as certain other substances, against drywood termites, with some comparative studies with other insect species.

Some of the silica aerogels have been impregnated with low percentages of certain inorganic fluorine compounds to enhance their value in specific industrial applications. This was found to have greatly increased their insecticidal effectiveness, in fact the impregnated or "coated" materials are much more effective than mechanical mixtures of the silica materials with the fluorine compounds ground to below 400-mesh, and even more effective than finely divided powders of the fluorine compounds themselves. Since the fluorine-impregnated materials were no less effective desiccants than the unimpregnated ones, they were included in the present investigation.

The prevention of attack on wood was the practical goal of the experiments. This phase of the investigation will be presented separately and in considerable detail in a companion paper by Wagner & Ebeling (pages 208 to 212).

EFFECT OF INERT DUSTS ON INSECTS.—Kühnelt (1928) showed that the ability of insects to withstand desiccation resulted from their possession of an epicuticular layer the outermost part of which was highly resistant to strong acids and contained fatty acids and cholesterols. Beament (1945) found the lipoid surface of the epicuticle to be approximately 0.25 μ thick in most of the insects he investigated. This would amount to 30 monolayers of wax molecules, if they were all vertically oriented. He believed, however, that only the lowest monolayer of molecules is completely oriented and tightly packed, and that this layer is the main barrier to the passage of water. This concept of the nature of the lowest monolayer of molecules is not shared by Hurst (1948) who believes that it is more probable that the wax at the epicuticular interface consists of "molecular aggregates in which there is random orientation." This type of wax layer he believes would exert a protective action on the underlying epicuticular "valve mechanism," modifying its sensitivity in accordance with the particular physiological requirements of the insect.

Beament found the pure extracted lipoids to be solid waxes, except in the case of the cockroaches, which have a "mobile grease." The hardest waxes were also the most

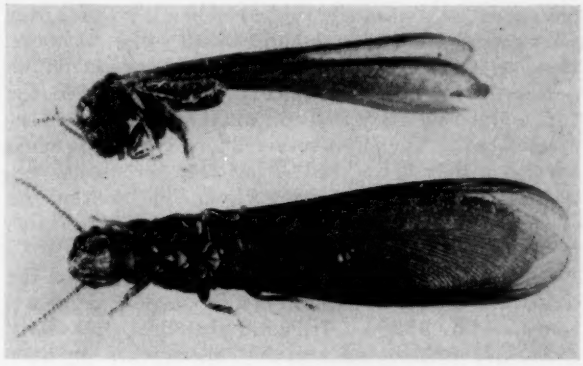

FIG. 1.—Above, drywood termite desiccated by means of a sorptive dust; below, untreated control.

hydrophobic. Sometimes a "cement layer" or "tectocuticle" issues from the dermal glands to cover the wax (Wigglesworth 1947, Richards 1951). Possibly this "cement" is shellac (Beament 1955). The evidence that the resistance of insects to desiccation is due to this layer of wax appears to be conclusive (Wigglesworth 1945, Beament 1945, Way 1950, Wolfe 1954, Holdgate & Seal 1956).

The majority of workers have concluded that inert dusts or powders, when they result in mortality of insects, are effective because of their ability to cause insects to lose water at an abnormal rate (Zacher & Künicke 1931, Hockenyos 1933a, Germar 1936, Zacher 1937a, b, Chiu, 1939a, Alexander *et al.* 1944, Kalmus 1944, Parkin 1944, Beament 1945, Wigglesworth 1945, 1957; Hunt 1947, Cotton & Frankenfeld 1949; David & Gardiner 1950, Bartlett 1951, Helvey 1952, Jones 1955, Holdgate & Seal 1956, Nair 1957). However, there have been a wide variety of opinions as to how the inert particles bring about this effect. Zacher and Künicke believed that dusts draw water out of the insects by capillarity, but this theory was considered untenable by Alexander *et al.* in view of the fact that they found hydrophobic dusts equally as effective as hydrophilic ones. Alexander *et al.* also discount the possibility that the inert dusts might desiccate insects by accumulating between the segments of the cuticle, exposing the more permeable intersegmental membranes, as suggested by Germar (1936). They point out that dusts can kill beetles even when they adhere in a layer not more than 1 μ thick; also that if loss of water depended on wedging the segments apart, increase in particle size up to a certain point should increase desiccation, whereas the opposite is true.

Hockenyos (1933a and b), Chiu (1939a), and Alexander *et al.* (1944) demonstrated that the inert dusts that cause desiccation do not affect the respiration of insects.

Wigglesworth (1945) and Beament (1945) found that inert dusts did not cause water loss of the motionless or dead insects they examined except for the cockroach and concluded that these dusts abraded the wax off the epicuticle, thus removing the principal barrier to the transpiration of water from the insect body. They believed that, for most insects, if adsorption is a factor in desiccation, it must be augmented by abrasion.

Wigglesworth (1945) observed that normally the abdomen of an engorged *Rhodnius* nymph touches the

ground and is continually rubbed. When it crawled over filter paper dusted with alumina it died within 24 hours. However, when a small amount of wax was placed on the portion of the abdomen touching the filter paper, the cuticle was held away from the alumina and did not become abraded. The insect survived for days despite being covered with a film of dust. When abraded insects were immersed in ammoniacal silver hydroxide solution the epicuticle stained deep brown only where the protective layer of wax had been removed.

Hurst (1948) took issue with the idea, prevalent at the time, that even if adsorption is a factor, it must be accompanied by abrasion. He noted that the permeability of the epicuticular "valve system" increases in the presence of water and that this is associated with an increase in mobility of the lipids at the lipid-protein interfaces. He concluded that the ability of abrasives to cause water loss should be increased under moist conditions, yet moist alumina particles did not cause water loss of *Tenebrio* larvae. When the dusted insects were washed and dried they still did not become desiccated when exposed to dry air. Hurst concluded that alumina caused water loss by adsorption only, and that water blocked the adsorptive surfaces of the dust particles.

Alexander *et al.* (1944) made important progress toward our understanding of the problem by distinguishing between the types of materials that are most effective against the relatively impermeable epicuticle of certain adult beetles and those that are most effective against the much more readily permeable epicuticles of beetle larvae. Against the granary weevil, *Sitophilus granarius* (L.), they found that the effectiveness of inert dusts increased with hardness of the particles and that a crystalline or angular surface was also desirable. The hardest particles, like those of diamond, carborundum and quartz, were very effective, the particles of talc, china clay and gypsum had little effect, and those of intermediate hardness, like fluorite and glass, were moderately effective. Effectiveness also increased with decreasing particle size down to ideal size of 1 or 2 μ. The same relationship was found by Chiu (1939*a*) and David & Gardiner (1950).

Against the red flour beetle, *Tribolium castaneum* (Hbst.), Alexander *et al.* (1944) discovered that powerful adsorbents, such as finely ground activated gas mask charcoal and a proprietary brand of calcined alumina, killed more rapidly than carborundum and quartz, although the latter were still effective. However, against the larvae of *Tribolium castaneum*, the yellow mealworm (*Tenebrio molitor* L.) and the Mediterranean flour moth (*Anagasta kühniella* (Zell.)), carborundum was no longer of any value, while the strong adsorbents (alumina and Neosyl) were highly effective.

Hunt (1947) investigated the lethal action of 61 inert diluents against the Mexican bean beetle, *Epilachna varivestis* Muls., and concluded that desiccation followed abrasion. It is worthy of note, however, that the pyrophyllites were among the least effective of these diluents and that Barden Clay was by far the most effective. The "abrasion index"[2] (loss in milligrams due to abrasion) of five representative diluents was found to be as follows: pyrophyllite 2900–3000, volcanic ash 1275–1325, attapulgite 758–800, talc 670–700, and Barden Clay 70–75, The insecticidal effectiveness of Barden Clay is clearly not the result of abrasion; it results from the small particle size and high absorbency of this material.

Cotton & Frankenfeld (1949) found silica aerogel more effective against the insect pests of stored grain than any other form of silica. They considered its effect in desiccating the four species of insects investigated the result of abrasion of the cuticle. However, silica aerogels are considered nonabrasive by the manufacturers. The key to its effectiveness was anticipated in the statement of the above authors that oil absorption by their silica aerogel was "too high for conventional test methods."

The delicate hymenopterous parasites might be expected to be particularly responsive to the action of sorptive dusts, and Bartlett (1951) found this true. He suggested the possibility that the dust particles might augment desiccation by the "disruption of an epicuticular wax film by sorption . . . ," but considered water-sorptive capacity the major factor in the ability of the dusts to desiccate the insects.

If the insect is protected by a continuous lipid film, however, it is difficult to envision the means by which a dust on its surface could draw water or vapor through that film. Although water loss from insects normally is proportional to the saturation deficiency of the atmosphere (Buxton 1932, Johnson 1942) and sorptive dusts could accentuate that deficiency at the body surface, it is not conceivable that such action would by itself result in the death of insects in as little as 2 or 3 hours, as in the case of drywood termites. They were found to survive for days in a calcium chloride desiccator when glued to a glass slide so as to prevent their normal tendency to huddle together to conserve moisture. It is likely that a disruption or removal of the lipoid protective film is required to bring about rapid desiccation.

Helvey (1952) showed by means of photographs made with the aid of an electron microscope that the most highly effective dusts, against the larvae of the Mexican bean beetle, were those that were least abrasive. Those with sharp edges and points had little or no insecticidal effect.

Jones (1955) produced evidence that strongly suggested that the water-proofing layer on the epicuticle of the worker honey bee contains a hard wax, probably similar to beeswax. This is the type of wax that was considered by most previous workers to require abrasion if rapid loss of water were to be initiated. Nevertheless, water loss brought about by dusting the bees with activated charcoal was half that which occurred when bees were immersed in boiling chloroform, which is believed to be able to completely extract any lipoid material on the surface of the cuticle. He proved that this relatively hard wax can be removed by adsorption alone, by dipping recently killed worker bees in a suspension of 1% (w/v) gamma grade alumina, containing also a wetting agent and stabilizer. After the bees were dried, their subsequent loss of water was 12.2 times greater than that of the controls which had been placed in the same solution minus the alumina.

When recently killed bees were exposed for 10 minutes to a dust cloud of activated charcoal with particles less than 1 μ in diameter, then placed in a desiccator, in the first 2 hours they lost water at a rate 30 times greater than

[2] Courtesy of J. M. Huber Corporation, New York. N. Y.

the controls. In 24 hours the average rate was 18 times greater. When living bees were exposed to a dust cloud for only a minute, not sufficiently long to result in their death, they lost water at a rate 4.1 times greater than the controls immediately after dusting but had recovered their impermeability completely 24 hours later (Jones 1955).

In the experiments by Jones (1955) the effect of dusts in desiccating bees appeared to be related to their sorptiveness. The three best materials were silica gel, Almicide and activated charcoal, all passed through a 300-mesh sieve. Among the least effective were such abrasive materials as carborundum and pumice powder.

Nair (1957) showed that sorptive dusts, such as finely powdered magnesite, kaolin and "paddy-husk" charcoal, could kill a number of species of beetles with a relatively hard and apolar wax as the waterprofing layer. He concluded that the effect of the inert sorptive dusts on insects is affected by the nature of the waterproofing layers. Insects with a "cement" layer covering the lipid material are more resistant than those with an exposed lipoid layer. Among the latter, those with hard and relatively apolar wax are more resistant than those with softer wax, with polar characteristics, which is more easily adsorbed.

METHODS.—Since among the termites the alate reproductives form the new colonies, preventive measures should be directed against them. Consequently they were used as the test insects in the present series of experiments whenever possible. To attain 100% mortality, the alates require, on the average, approximately 70% as long a period of contact with desiccating dusts as the full-grown nymphs. Alates were obtained by placing infested walnut wood in a cage and using the phototropic reaction of these insects to trap them in large numbers during the normal swarming periods in the fall.

At other periods the full-grown nymphs were used as test insects. They can be obtained at any time of the year by splitting open the dead limbs of walnut trees and tapping the split portions over a suitable receptacle. The termites and pellets that tumble from the opened galleries may be shaken on a screen to get rid of the pellets. The termites, together with bits of bark and debris not passing through the screen, may be placed on top of several layers of paper towelling, with other layers of towelling placed over them. The uninjured termites will crawl away from the original mass and congregate in large masses between pieces of paper. From these locations they can be removed to petri dishes containing about a dozen layers of paper towelling, which they use as food.

Toxicity evaluations were usually made by placing the termites on films or layers of dust that were sufficient in quantity to insure that the insects became well covered with the dust, thus testing the intrinsic lethality of the dust rather than its practical usefulness under field conditions. Ten termites were placed in either two or three containers for each material tested. These containers might be stender dishes 1 inch in diameter, which could be covered with glass covers for the maintenance of a water-saturated atmosphere, or they might be 100-ml. beakers or jelly jars with a bottom area of $1\frac{1}{2}$ or 3 square inches. Fortunately the alates cannot fly when confined in small areas.

In one experiment the jelly jars were placed in desiccators in which various humidities were maintained by means of differing sulfuric acid concentrations. In another experiment termites, as well as other insects, were placed in a dusted wooden cage

Comparisons of the effectiveness of different dusts were usually based on the period required for 50% mortality (MLP), for 100% mortality, or for both. A termite was considered to be ' dead" when no further movements of the appendages could be elicited by gentle probing with a needle.

In experiments on weight loss from desiccation, the termites were either gently dusted or shaken with the dust, the excess dust was then separated from the termites by shaking them on a screen and the termites were then weighed on 1-inch wide celluloid vials not more than an inch in height. In other experiments the termites were left on layers of dust in beakers or jars for the prescribed period, then the excess dust was removed before weighing. The termites were returned to the dust, between weighings, if several weighings were made.

INSECTICIDE MATERIALS.—The five most promising inert dust diluents, based on preliminary tests with 160 materials dusted on wood blocks, as well as pyrophyllite, quicklime and slaked lime, silica products, a number of abrasives and adsorbents, and insecticide dusts or powders were tested for their effectiveness against drywood termites.

Certain materials, such as the botanical dusts, most of the talcs, and sulfur, were of no value in the preliminary tests, presumably because they were neither abrasive nor sorptive. Therefore they were not used in the following tests despite their commercial importance as diluents.

The silica products used in this investigation proved to be of such interest that a discussion of their composition and properties seems appropriate at this point. The silica aerogels and silica precipitates are finely divided silica products for preparing "flatted" lacquers. The aerogels are formed when sulfuric acid is added to sodium silicate, to produce a firm "hydrogel." This is washed free of reaction salts and dried to form a granular dehydrated silica aerogel that can be reduced to very small particle size. Despite the small size of the final particles, they are porous. Air replaces the liquid in the hydrogel without otherwise altering its structure. Such characteristics as pH, moisture content, surface area, oil adsorption, and density can be controlled. If the silicate is added to the acid, a "silica precipitate" is formed. It has less sorptive capacity than the silica aerogel.

Of the silica aerogels used in the present experiments, SG 77 has a density (centrifuge) of 0.30 and an average particle size of 7 μ. It is prepared the same as SG 975 except for impregnation with magnesium silicofluoride so as to result in a fluorine content of 1.6% in weight. The theoretical percentage of magnesium silicofluoride is 3.85. SG 67 is a silica aerogel with a density (centrifuge) of 0.20 and an average particle size of 3.5 μ. A pound of SG 67 can adsorb 2.55 lbs. of oil compared with 1.70 lbs. for SG 77. SG 67 is formed by impregnating SG 68 with ammonium silicofluoride so as to result in a fluorine content of 3% by weight. The theoretical percentage of ammonium silicofluoride is 4.7. The exact percentages of the above compounds are not known, for there is probably some decomposition during the drying of the hydrogel in

the process of manufacture.

The fluorides are incorporated into the silica particles by treating the silica hydrogel with these compounds until the fluorine content is the desired amount, on a dry basis. The principal reason the fluorine compounds are added, from the standpoint of industrial use, is that they prevent the silica aerogel from forming a hard cake when dried.

Even though the silica particles are extremely small, they are themselves porous, and consequently it has been calculated that the fluorides occur on the particles in somewhat less than a continuous monomolecular layer.

The dusts and powders used in the experiments were as follows:

Alumina—1557 AB levigated alumina powder
Anhydrone—Anhydrous magnesium perchlorate
Attaclay—highly sorptive attapulgite
carbon—decolorizing
carborundum—silicon carbide, 500 mesh
chlordane—5% in pyrophyllite
Desicchlora—anhydrous barium perchlorate
Dieldrin—99.5% technical
Diluex A—highly sorptive attapulgite
Hi-Sil—silica percipitate
lime—quicklime
lime—slaked
Norit—acid washed, charred, walnut shell flour
Olancha Clay—highly sorptive montmorillonite
Olancha Clay—micronized
parathion—25 per cent wettable powder
Pike's Peak Clay—highly sorptive montmorillonite
pyrophyllite—Phyllite
sand—powdered with glass mortar and pestle
Santocel C—silica aerogel
Santocel FRC—silica aerogel
SG 67—SG 68 impregnated with a fluoride
SG 68—silica aerogel
SG 77—SG 975 impregnated with a fluoride
SG 975—silica aerogel
Sierra White I. R. talc—low sorptive talc, 200 mesh
Sierra Mistron white talc—high organic sorptive talc, maximum particle size 6μ
sodium fluoride—technical 95%, powdered
sodium pentachlorophenate—powdered

EFFECT OF SORPTIVE MATERIALS AGAINST DRYWOOD TERMITES.—In the first experiment, alate termites were either gently rolled about or vigorously shaken 10 times (Series C) with 1 cc. of dust in 2½-inch glass vials, then shaken on a screen to remove excess dust, and placed in 1-inch stender dishes. In all tests the excess dust was removed from the termites in less than 5 minutes and they were then placed in the dust-free stender dishes.

In Series A, B, and C (table 1) there was a duplicate set of three stender dishes for each material which had moist paper at the bottom so as to completely saturate the atmosphere, as long as the ground-glass covers were kept in place. Most of the results of the first experiment are shown in table 1, but the data for the termites kept in a moisture-saturated atmosphere are not included except for treatments 8, 9, 12, 38 and 40, for the inert or unimpregnated diluents did not kill termites kept under such conditions.

The lower MLP's, with the same materials, in Series C, as compared with Series A and D, are believed to be due to the shaking of the termites with the dust in Series C,

Table 1.—Period required for 50% mortality (median lethal period) of drywood termites dusted with inert powders and insecticides.

TREATMENT NUMBER	MATERIAL	MLP (HOURS)
	Series A[a]	
1	Olancha Clay	3.0
2	Pike's Peak Clay	4.6
3	Attaclay	5.0
4	Diluex A	4.6
5	Cox's Earth	7.1
6	pyrophyllite+5% lindane	3.5
7	NaF powder	4.5
8	NaF powder[b]	5.1
9	sodium pentachlorophenate[b]	3.5
	L.S.D. at 5% level	0.4
	Series B	
10	pyrophyllite	14.7
11	pyrophyllite (ground)	10.2
12	pyrophyllite+5% lindane[b]	46.7
13	sodium pentachlorophenate	16.2
	L.S.D. at 5% level	3.8
	Series C	
14	Olancha Clay	1.7
15	Olancha Clay+5% parathion	1.7
16	Olancha Clay+5% NaF	1.8
17	Olancha Clay+5% Paris green	1.9
18	Olancha Clay (micronized)	1.7
19	Olancha Clay with 1 ml. oil per 5 g.	2.0
20	Sierra Mistron Vapor	2.7
21	Sierra White I.R.	3.0
22	pyrophyllite	8.4
23	pyrophyllite+5% NaF	10.0
24	pyrophyllite+25% parathion	2.2
25	Hi-Sil	1.4
26	Santocel C	1.4
27	lime (quicklime)	1.5
28	lime (slaked)	3.8
29	Anhydrone	1.8
30	Alumina	10.5
31	carborundum	4.7
32	sand (powdered)	2.6
33	decolorizing carbon	1.6
34	acid washed norit	5.5
35	carborundum and Alumina	3.7
36	carborundum and decolorizing carbon	2.4
	L.S.D. at 5% level	0.7
	Series D	
37	SG 67 (coated silica aerogel)	1.6
38	SG 67[b]	24.0
39	SG 77 (coated silica aerogel)	1.7
40	SG 77[b]	72.0
41	SG 68 (silica aerogel)	1.8
42	Santocel FRC	1.9
43	Olancha Clay	2.0
	L.S.D. at 5% level (except for tests 38 and 40)	0.2

[a] Date of application and temperature and humidity during the first 5 hours for the three series of tests are as follows: Series A and B, September 26, 1957, temperature 74 to 76° F., relative humidity, 54%; Series C, October 2, 1957, temperature 73 to 76° F., relative humidity, 58%; Series D, June 8, 1958, temperature 78° F., relative humidity, 49%.

[b] The termites, after initial treatment, remained in an atmosphere saturated with water vapor throughout the experiment.

allowing for contact of a greater number of dust particles with the termite bodies. In A and B the termites treated with inert diluents and kept in the saturated atmosphere survived as long as the covers were left on the dishes. The dust particles gradually fell from the bodies of the termites, but were not picked up again in the moist environment. No desiccation took place. However, all the termites in the moisture-saturated atmosphere died after the covers of the stender dishes were removed, which was

24 hours after treatment. In Series D the covers on the stender dishes containing the water-saturated atmosphere were left on for 3 days. No mortality occurred in the water-saturated stender dish accompanying treatment Nos. 37, 39, 41, 42 and 43. However, in treatment No. 38 there was an 80% mortality in 8 hours and 100% in less than 1 day. In treatment No. 40 there was an 80% mortality in 3 days. The materials used in these tests had been impregnated with fluorine compounds which were effective under moist conditions.

In a further testing of the effects of a water-saturated atmosphere, not indicated in table 1, three lots of termites were treated with Olancha Clay, three with pyrophyllite, and three were left untreated. They were all kept in moist stenders. As before, the termites became quite free of dust. However, the treated ones were not as active as the untreated ones and some showed unmistakable signs of distress or were moribund, indicating that the presence of dust on an insect may have an adverse effect aside from that resulting from desiccation. Chiu (1939b) arrived at the same conclusion as the result of his investigations with inert materials used against rice weevils and granary weevils. In the present investigation the moribund termites were removed from the dishes and are not considered in the following data.

After the covers of the moist stender dishes were removed 1 day after treatment, the MLP's were as follows: Olancha Clay 11 hours, pyrophyllite 27 hours; after 2 days Olancha Clay 18 hours, pyrophyllite 24 hours; after 3 days no mortality in 24 hours for either Olancha Clay or pyrophyllite. It was evident that the damage done by the dusts was gradually repaired over a 3-day period in a water-saturated atmosphere.

In treatment No. 12 (table 1), a period of 46.7 hours was required for 5% lindane in pyrophyllite to effect a 50% kill of termites, compared with 3.5 hours when the termites were left in the normal atmosphere (treatment 6). In the moist atmosphere the diluent had, for the time being, no effect, and the high resistance of termites to even such a relatively highly effective toxicant as lindane was demonstrated. On the other hand, moisture increased the effectiveness of sodium fluoride and sodium pentachlorophenate powders, particularly the latter, for the insecticide was brought into solution in the condensed liquid on the insects' bodies (treatments 8 and 9). Besides, the bodies of the termites imbibe water, along with the water-soluble toxicants, as shown by their tendency to swell when they are in contact with moisture.

In Series A, lindane increased the effectiveness of pyrophyllite under dry conditions. However, pyrophyllite alone is relatively ineffective and it is understandable that its effectiveness might be increased by the addition of a toxicant. In Series C the insecticides (sodium fluoride, Paris green and parathion) added to Olancha Clay did not increase its insecticidal effectiveness because the termites became desiccated so rapidly that the effects of the toxicants *per se* could not be measured. The typical paralysis caused by parathion was evident before the termites died, but they died no sooner from having suffered this paralysis. Normally the period between onset of paralysis and the death of the insects is greatly prolonged in the case of termites treated with chlorinated hydrocarbons and organophosphorous compounds. Note that in treatment 24 the MLP was longer for 25% parathion in pyrophyllite than for Olancha Clay alone, presumably because pyrophyllite causes desiccation of the termites less rapidly than Olancha Clay.

Table 2.—Effects of the nature of the diluent on the lethality of 5% dieldrin dusts at different relative humidities.[a]

Material	Date (1958)	Relative Humidity (%)	MLP (Hours)
SG 67	June 21	68	2.2
SG 67+dieldrin		68	1.9
Olancha Clay		68	3.1
Olancha Clay+dieldrin		68	2.8
Pyrophyllite		68	8.3
Pyrophyllite+dieldrin		68	7.9
Walnut shell flour		68	47.0
Walnut shell flour+dieldrin		68	30.0
Recrystallized dieldrin		68	32.0
SG 67	June 22	60	1.5
SG 67+dieldrin		60	1.6
Olancha Clay		60	2.1
Olancha Clay+dieldrin		60	2.1

[a] Temperatures ranged from 74 to 76° F. during the first 3 hours of the experiment on both days of the experiment.

In another test in which the MLP for termites dusted with Olancha Clay was 1½ hours, it was 5 hours for termites placed on natural cryolite, 15 hours for termites on the amorphous, waxy, technical DDT, 18 hours for termites on lindane crystals, and 28 hours for termites on the needle-like crystals of pure dieldrin, with which the insects were constantly covered.

The great difference in the effectiveness of a 5% lindane dust at 54% and at 100% relative humidity (table 1) suggested the advisability of a more direct investigation of the influence of the diluent on insecticidal effectiveness. Dieldrin was chosen as the toxicant because of the ease of incorporation of the pure, recrystallized product into a diluent by means of a ball mill. An experiment was made in which 0.5 cc. of a diluent with or without 5% dieldrin was placed in a 100-ml. beaker together with 10 large drywood termite nymphs. Three beakers were used for each material and each beaker was shaken for a moment to insure the immediate coverage of the termite bodies with dust. The temperature during the first 3 hours of the experiment—the period required for the two most effective diluents to give a 50% kill—ranged from 74° to 76° F. The relative humidity during this period was 68% on the first day of the experiment and 60% on the following day. The results are shown in table 2.

Table 2 shows that dieldrin itself kills drywood termites very slowly. However, it decreases the MLP of walnut shell flour, one of the least effective diluents, by over 50%. Its ability to decrease the MLP of more effective diluents decreases with increasing effectiveness of the diluent, but in all cases the MLP of the 5% dieldrin dust is determined primarily by the nature of the diluent rather than the toxicant.

On the second day of the experiment the relative humidity was only 60% when the temperature had reached 74° F. Under this less humid condition, the dieldrin no longer increased the effectiveness of the diluents, indicating that, as the humidity approaches the optimum for

the more effective diluents, they themselves kill so rapidly that there is insufficient time for toxicity *per se* to exert even the slight effect noted at higher humidities. To further substantiate this conclusion, it will be recalled that in table 1 the MLP for Olancha Clay plus 5% parathion was no different than for Olancha Clay alone when the relative humidity was 54% (Nos. 14 and 15).

In the experiments reported in this paper, the test insects have generally been treated in such a manner, or with such a quantity of powder, that the intrinsic value of the powder at an optimum deposit on the insects' bodies has been evaluated. Such practical factors as the type of dust cloud the material would form, its ability to deposit well on the insects' bodies, its adhesiveness, etc., cannot be measured in this way. This is not meant to imply, however, that a thin film of powder would not be effective, at least against drywood termites. Practical experience in the dusting of attics for the prevention of infestation by the alate reproductives has shown that their exposure to a very thin film of powder will result in reasonably rapid mortality. A barely perceptible film of the fluorine-coated silica aerogel SG 78 on the undersides of rose leaves can kill two-spotted spider mites, *tetranychus telarius* (L.), according to Dr. R. N. Jefferson.

To obtain comparative data on the effectiveness of extremely thin films as compared with thick layers of powder, an experiment was made in which drywood termite nymphs were placed on both types of deposits. These deposits were applied to the bottoms of jelly jars having a bottom area of 3 sq. inches. In one series of jars black photographic paper was glued to the bottoms so that the appearance of thin films of dust could be observed visually as they settled on the paper after being released in bell jars. The papers were later removed and the deposits were weighed. In the other series of jars 1 cc. of powder was weighed and was then evenly distributed over the bottom of each jar. For each material and each type of deposit, 10 drywood termite nymphs were placed in each of three jars. The temperatures during the first 4 hours of the experiment ranged from 82° to 84° F. and the relative humidity during this period was 60%.

The materials and weights used per jar were as follows for the heavy deposits: SG 68 and SG 67, 120 mg. and SG 975 and SG 77, 270 mg.; for the light deposits, SG 68, 0.8 mg.; SG 67, 0.7 mg.; SG 975, 1.5 mg.; and SG 77, 1.7 mg. There were no significant differences in the effectiveness of the various materials, but the period for 100% mortality averaged 1.6 hours for the heavy deposits and 3.4 hours for the light deposits. The experiment was repeated with drywood termite alates under practically identical conditons of temperature and humidity, and the corresponding ratio was 1.4 hours to 2.7 hours.

Although the ratio of effectiveness of heavy layers of dust compared with extremely light deposits, with termites as the test insects, is only approximately 2 to 1, it should not be inferred that heavy deposits are not desirable. In the present experiment the termites were confined continuously with the deposits. In the control of some insects, like cockroaches, the desiccating powders would be applied only to limited areas such as behind stoves, under refrigerators and sinks, in pantries, etc. After a brief contact with the powder the cockroaches might crawl off to untreated areas and the quantity of powder picked up during the brief contact would have to suffice. Also the value of a heavy deposit of powder might be relatively greater with the larger insects.

RELATION OF WEIGHT LOSS AND MORTALITY.—Table 3 shows the loss in weight of lots of 10 termites shaken in vials with four types of dust. It appeared from observation that slaked lime resulted in the heaviest deposit of dust on the bodies of the termites and Olancha Clay the least. The temperature at the beginning of the experiment was 76° F. and the relative humidity 56%. The periods required for 5 out of 10 termites to die as the result of their contact with the various dusts were as follows: Olancha Clay, 1.75 hours; pyrophyllite, 6 hours; ground pyrophyllite, 3½ hours; and slaked lime, 4 hours. A 50% mortality of the termites can thus be seen to have occurred by the time the loss of water ranged between 30% and 35%, regardless of the type of dust used.

COMPARATIVE TESTS WITH VARIOUS INSECT SPECIES.—Wigglesworth (1945), using dead cockroaches to avoid possible effects of abrasion, and dusting them with Alumina, a highly sorptive dust, stated that " . . . adsorption by the dust quickly leads to increased evaporation. The high degree of mobility in the waterproofing grease of the cockroach not only leads to its removal by adsorption, but . . . it allows a considerable amount of recovery in the dead insect—by the spreading of the oil over the abraded areas."

The termites are considered to be taxonomically somewhat related to the cockroaches. Jeannel (1949) places them in the same super-order, the Blattopteroides. The similarity in the superficial appearance of the cuticle is particularly striking in these two groups. Ramsay (1935) showed that a lipoid film will form on droplets of water sprayed on cockroaches. The mobile "grease" on the epicuticle of the cockroach was found by Beament (1955) to consist of a hard wax (m.p. 56° C.) dissolved in a "solvent" which is liquid at room temperatures. The grease may consist of equal amounts of wax and solvent. Beament believes that the short-chain molecules of paraffins and alcohols comprise the solvent while the molecules of longer chain lengths comprise the wax.

Patches of oily film were likewise seen when droplets of water were placed on alate drywood termites, but in lesser quantity than for the cockroach. The oily material was more apt to be in the form of many circular lenses rather than in large, irregular films. This indicates that a portion of the lipoid protective layer of the termite consists of an oil with polar characteristics, but to a lesser extent than in the case of the cockroach.

The presence of oily liquid having been demonstrated, the action of sorptive dusts in removing a substantial

Table 3.—Loss in weight of lots of 10 live termites dusted with various diluents.

MATERIAL	PER CENT DECREASE IN WEIGHT DURING HOURS INDICATED			
	0–2	2–4	4–6	0–24
Olancha Clay	33.3	19.1	5.0	59.6
Pyrophyllite	15.0	10.0	10.0	53.0
Pyrophyllite (ground)	16.3	16.3	11.9	59.8
Slaked lime	19.4	12.4	9.3	57.7

quantity of the protective lipoid layer on the epicuticle of the termite is readily understandable. However, the possibility of the adsorption of not only oil, but also wax, by non-abrasive materials is not necessarily precluded. Silica aerogel, stated by the manufacturers to be completely non-abrasive, is said to cause the death of granary weevils by desiccation and was found by the writers, in the ammoniacal silver hydroxide staining test, to remove the lipid layer of saw-toothed grain beetles, *Oryzaephilus surinamensis* (L.).

An experiment was made to determine the effect of various sorptive dusts on two insects that differed from cockroaches and termites in apparently having no lipid material of an oily or greasy nature on their epicuticle, namely, the vinegar fly (*Drosophila melanogaster* Meig.) and the house fly (*Musca domestica* L.). The periods required for 50% (termites and vinegar flies) and 100% (cockroaches and house flies) "knockdown" and mortality were determined. The results are shown in table 4. Those pertaining to cockroaches were obtained from Dr. I. B. Tarshis.

Table 4.—Period required for 50% (termites and vinegar flies) and 100% (cockroaches and house flies) knockdown and mortality with sorptive dusts and certain insecticides.[a]

Material	Period (Minutes) Required for	
	Knockdown[b]	Mortality
Drywood termite (76° F., R.H. 74%)		
SG 77	17	67
SG 67	22	83
Olancha Clay	40	94
Lindane 25W[c]	9	163
German cockroach (78–83° F., R.H. 42%)		
SG 77	20	60
SG 67	15	45
Lindane 12D[c]	15	105
Chlordane 50W	240	540
Dieldrin 50W	60	165
Pure lindane	30	600
Diazinon 25W	45	165
Pure Dipterex	165	450
Vinegar Fly (75° F., R.H. 75%)		
SG 77	25	34
SG 67	28	39
Olancha Clay	70	98
Lindane 25W	11	60
House fly (81° F., R.H. 80%)		
SG 77	63	72
Lindane 25W	55	220
DDT 75W	57	296
Chlordane 50W	182	360
Dieldrin 50W	186	397
Parathion 25W	23	320
Malathion 25W	38	328
Diazinon 25W	50	308

[a] The experiments were made in April, 1958. Weighed or measured amounts of dust were placed on the bottoms of glass jars as follows: for termites, 0.45 cc. per 2.73 sq. in.; for cockroaches, 0.5 gm. per 5 sq. in.; for vinegar flies, 0.04 cc. per 2.73 sq. in.; and for house flies, 1 cc. per 2.73 sq. in. The insects were placed in the jars to crawl about on the dusts. The jars were covered in the tests with vinegar flies and house flies. The great difference in bulk density of the silica aerogels and the other common diluents is indicated by the following weights of the 0.45 cc. of material used in the termite experiments: lindane 25W, 192 mg.; Olancha Clay, 161 mg.; SG 77 , 49 mg.; and SG 67, 18.7 mg.

[b] "Knockdown" was considered to be the point at which the insect was on its back and no longer mobile. For both "knockdown" and "mortality" the period when 50% of the insects were affected was recorded for termites and vinegar flies and 100% for cockroaches and house flies.

[c] The letters W and D refer to the type of formulation, wettable powder or dust, and the numbers indicate the per cent of toxicant.

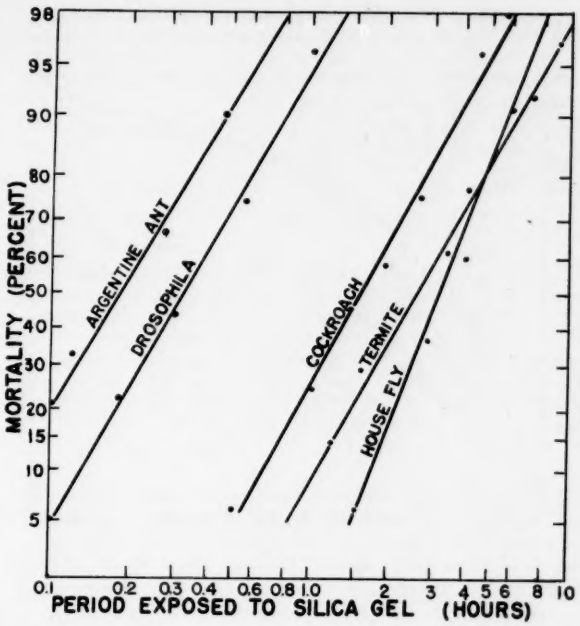

FIG. 2.—Dosage-mortality regressions for five species of insects confined in a cage dusted with silica aerogel SG 77.

Table 4 shows that lindane resulted in more rapid knockdown than the silica gels or aerogels except that against the German cockroach (*Blattella germanica* L.) it acted no more rapidly than SG 67. However, for all four species of insects, the silica products killed more rapidly than any of the toxicants. The ratio of knockdown time to kill time was much less for the materials that are effective desiccators than for those that kill primarily by toxic action.

Dosage mortality regressions for the above four species, plus the Argentine ant, *Iridomyrmex humilis* Mayr, were determined. Two grams of SG 77 were blown into a wooden rearing cage of a volume of 5.6 cu. ft. and an inside surface of 18 sq. ft. The dust was circulated in the cage for 5 minutes by means of an electric fan to insure a uniform deposit. The insects were liberated in the cage, the ants being placed in petri dishes that were in the cage when it was dusted. The vinegar flies and house flies crawled about primarily on the vertical walls until they were immobilized by the treatment, whereas the cockroaches, ants and termites remained on the floor of the cage. This experiment makes possible a comparison of five species under the same conditions of treatment. The results are shown in figure 2.

The periods for 50% kill, in minutes, were as follows: ants 10, *Drosophila* 19, German cockroaches 92, drywood termites 152, and house flies 185. The steeper curve for the house flies is probably caused by the uniformity of the age of the flies; they were all of an adult age of 4 days.

An experiment was made to determine whether the rate of water loss of cockroaches, termites, and house flies was related to the period required to kill these insects by means of sorptive dusts. Four male cockroaches, 10 termites, or 40 flies, the latter anesthetized with carbon dioxide, were shaken 10 times in a $\frac{3}{4}$- $\times 3\frac{1}{4}$-inch vial in which was placed 0.5 cc. of dust. The excess dust was re-

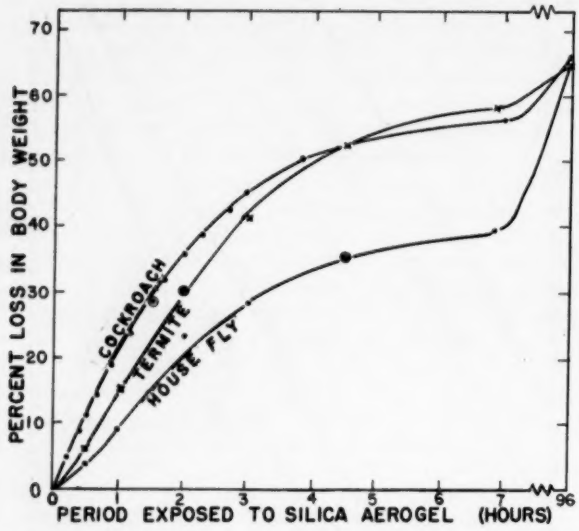

FIG. 3.—Loss of body weight of three species of insects with increasing period of exposure to silica aerogel SG 67. The encircled points indicate the periods required for 100% mortality.

moved by shaking the insects on a screen. The dusts used were SG 67, SG 68, and Olancha Clay. The temperature at which the insects were kept was 76° F. and the relative humidity was 60%.

The cockroaches were impaled on a No. 1 pin stuck into a No. 4 cork; the termites were placed in a light plastic vial 1 inch in diamter and 1 inch in height; and the flies were placed in the same kind of vial, but covered with a plastic screen. The insects were weighed periodically to determine the rate of water loss. This is graphically depicted as per cent loss in body weight with increasing period of exposure in figure 3, using only the data for SG 67 for purposes of illustration. In the figure, the encircled points indicate the period that had elapsed for a complete kill.

Cockroaches treated with SG 67 suffered a 100% mortality in 1.5 hours, with a water loss of 28.6% of their body weight; the termites reached the 100% mortality point in 2 hours, with a water loss of 30.2% of their body weight; and the house flies, which required 4.5 hours for 100% mortality, had by that time lost about 35% of their body weight in water. When these three species had become completely desiccated, they had all lost approximately 65% of their body weight.

EFFECT OF RELATIVE HUMIDITY.—Table 1 shows that, with the exception of SG 67 and SG 77, the sorptive dusts investigated had little effect against drywood termites if the latter were placed in a water-saturated environment immediately after treatment and for a long enough period to allow them to replace the epicuticular lipids. Since the inert dusts appear to kill insects primarily by desiccating them, it might be expected that their insecticidal effectiveness might be inversely proportional to the relative humidity of the atmosphere, other things being equal.

An experiment was made in which three lots of 10 drywood termites each were placed on blocks of wood dusted with an unimpregnated silica aerogel (SG 68) and three lots of 50 *Drosophila* were placed in 4-ounce jars, the insides of which had been lightly dusted with the same material. These tests were first made at 80° F. and 72% relative humidity and the following day at 80° F. and 44% relative humidity. The insects were placed on the same treated surfaces on both days. At the higher humidity the termites required 370 minutes for complete mortality and at the lower humidity 260 minutes, a decrease of 30%. The corresponding figures for the *Drosophila* were 280 and 165, a decrease of 41%.

In tests with house flies, 2 grams of SG 68 was dusted into a wooden rearing cage with a volume of 5.6 cu. ft. and an inside surface of 18 sq. ft. Fifty house flies were placed in the cage at a temperature of 76° F. and a relative humidity of 61% and in a second test 50 flies were exposed to the same residues but at a temperature of 75° F. and at a relative humidity of 32 to 35%. Dosage mortality regressions showed that 50% of the flies died in 7.6 hours at the higher humidity and in 4.5 hours at the lower humidity.

The fact that, in the experiment depicted in table 1, SG 67 and SG 77 were able to continue to kill termites in a water-saturated atmosphere, indicated the desirability of obtaining further evidence on this unique characteristic. Relative humidites of 20, 60, 90, and 100% were maintained in four 9-inch-wide glass desiccators by means of the appropriate concentrations of sulfuric acid. Twenty drywood termite nymphs were placed in a glass jar with a bottom surface of 3 sq. in. and containing 1 cc. of powder and then placed in the desiccator. In each of the 4 desiccators there were 6 such jars containing the 6 powders used in the experiment and 1 jar with no powder, used as the check. The jars were taken out of the desiccators and the termites were examined with the aid of a stereoscopic microscope when it appeared from periodic gross inspections that bodily movements had ceased.

Within 6 hours all termites placed in SG 67 had died at all relative humidites, and all termites had died at 60% relative humidity with SG 68 and Olancha Clay. It was decided at that point to weigh all the termites. They were placed on a screen to sieve off the excess powder, then brushed with a camel's-hair brush to remove as much of the powder adhering to the body as possible. The amount remaining was too small to have any appreciable effect on the calculated weight loss from desiccation. The results of the experiment are shown in table 5. The ability of the powders to desiccate the termites varied from most to least effective as follows: SG 67 > SG > 68 > Olancha Clay > pyrophyllite plus 5% chlordane > pyrophyllite > ammonium silicofluoride. The brand of pyrophyllite used in the 5% chlordane dust was not known. It is possible that the difference in the desiccating ability of 5% chlordane and the pyrophyllite alone could have been the result of differences in the physical nature of the pyrophyllites.

The net loss of weight from desiccation in water-saturated atmosphere was particularly noteworthy. It is obvious that drywood termites have little ability to protect themselves against desiccation under the conditions of this experiment. In nature they appear to be able to protect themselves against desiccation, at low humidities, by huddling together in a compact mass to reduce evaporation to the minimum and to seal themselves away from the adverse environmental influences by means of a pro-

Table 5.—Loss of body weight of drywood termite nymphs from desiccation at various constant relative humidities.[a]

Material	Per Cent Relative Humidity	Per Cent Loss in Body Weight in 6 Hours	Material	Per Cent Relative Humidity	Per Cent Loss in Body Weight in 6 Hours
SG 67	20	70.4	Pyrophyllite +5% chlordane	20	57.3
	60	55.2		60	45.0
	90	43.1		90	30.4
	100	36.7		100	24.0
SG 68	20	66.2	Pyrophyllite	20	42.3
	60	51.8		60	35.6
	90	40.3		90	26.5
	100	27.3		100	12.2
Ammonium silicofluoride	20	26.6	Untreated	20	21.0
	60	22.8		60	10.2
	90	10.4		90	2.3
	100	8.2		100	2.0
Olancha Clay	20	63.6			
	60	54.3			
	90	36.9			
	100	29.0			

[a] Temperatures ranged from 75° to 77° F.

tective carton of wood bits and fecal matter (Pence 1956).

Another experiment was made in which the four relative humidities, 20, 60, 90 and 100%, were maintained in the same way as in the previous experiment and the objective was to determine the period for 100% mortality. Drywood termite alates were available so they were used instead of nymphs. The powders were placed in 3-square-inch jars as before. They were the same as those used in the previous experiment except for Olancha Clay. Ten termites were placed in each of two jars for each powder and at each of the four relative humidities. The results of the experiment are shown in table 6.

A range of relative humidities from 20 to 100% had little effect on the rate of mortality of the termites placed in SG 67, whereas with SG 68, the same material except for the fluoride impregnation, the period for 100% mortality ranged from 1.1 for 20% relative humidity to 7.5 for 100% relative humidity. Even the higher figure indicates a remarkable degree of insecticidal effectiveness, however, when compared with another diluent, pyrophyllite, in which the termites remained alive for an average of 88.5 hours.

The relatively rapid death rate with both impregnated and unimpregnated powders, when compared with the previous experiment, in which it required from 4 to 6 hours to obtain a 100% mortality of the termites in the SG 67, can be accounted for by the higher temperatures at which the experiment was conducted, 82 to 83° F., compared with 76° to 78° in the previous experiment, and also the fact that alates were used in the present experiment instead of nymphs. As stated before, the alates are more easily killed, both by desiccants and by toxicants. The more rapid kills in the water-saturated atmosphere, when compared with the results in table 1, however, require further explanation. In the experiment the results of which are shown in table 1, the termites were freed of excessive powder before they were placed in the stender dishes. They had a tendency to lose the remaining powder without again picking it up from the damp substrate. In the present experiment the termites were in constant contact with a thick layer of powder.

Table 6 shows that SG 67 and its "coating" material, ammonium silicofluoride, were the least adversely affected by increasing humidities, toxicity obviously increasingly predominating over desiccation as a mortality factor as the relative humidity was increased. The ability of SG 67 to kill insects much more effectively than the fluorine compound with which it is impregnated has proved to be of great practical significance and will be discussed further in a later section of this paper.

Table 6.—Effectiveness of impregnated and unimpregnated dusts at various relative humidities against alate drywood termites.[a]

Material	Period (Hours) for 100% Mortality at Relative Humidities of 20%	60%	90%	100%
SG 67	0.75	0.85	0.96	1.0
SG 68	1.1	1.7	2.7	7.5
Ammonium silicofluoride	2.0	3.6	3.8	4.0
Pyrophyllite+5% chlordane	1.9	2.2	3.0	9.2
Pyrophyllite	2.2	3.5	10.2	88.5

[a] For each material and humidity, 10 termites were placed in each of two glass jars with a bottom surface of 3 sq. in. and containing 1 cc of powder. Temperatures ranged from 82° to 83° F. during the first 4 hours of the experiment and from 72° to 83° F. during the entire experiment.

At the lower humidities the advantage of SG 67 over SG 68 was much greater than in the experiments made in a ventilated room (see table 1). The stationary air in the desiccators is not as conducive to rapid desiccation as is the circulating air, at the same temperature and humidity, in an open room. It places those materials which depend on desiccation alone, for insecticidal effect, at a disadvantage. For the same reason it was found undesirable to allow desiccating termites to remain in ½" to 2" celluloid vials, for convenience in weighing, in the experiments on the relation of water loss to mortality. The lack of air circulation in the vials greatly increased the ratio of effectiveness, for example, of such materials as SG 67 and SG 68, to the disadvantage of the latter.

It is interesting to note that a 5% chlordane dust was highly effective at 20% relative humidity, but that this effectiveness may be attributed primarily to its diluent, pyrophyllite, which happens to be relatively effective at low humidities. The effectiveness of this diluent greatly decreases with each increase in humidity, until at 100% relative humidity it contributes practically nothing to the effectiveness of the chlordane. However, the brand of diluent with which the chlordane was mixed in the present experiment may have been somewhat more effective, for, within the 9.2 hours required for the chlordane dust to give a 100% mortality, at 100% relative humidity, the lethal amount of water loss (30 to 35%) of body weight may have been reached, if we may use the figures from table 5 as a criterion.

All the desiccating dusts caused a surprising degree of water loss in 100% relative humidity. Insects are apparently able to lose water in a water-saturated atmosphere if the lipid layer is removed or adversely affected. Possibly also the sorptive dusts are able to adsorb free water after they have reached their equilibrium with a water-saturated atmosphere. This might be expected when it is considered that dusted insects will exude droplets of

Table 7.—Comparison of rates of mortality of drywood termite nymphs on layers of dry powders and in slurries of the same dusts.

Material	Period for 100% Mortality	
	On Dry Powders[a] (Hours)	In Slurries[b] (Hours)
SG 68	3.1	>96.0
SG 67	2.9	2.0
SG 975	3.0	>96.0
SG 77	3.0	2.0
Ammonium silicofluoride	8.9	1.4
Magnesium silicofluoride	>24.0	2.2
Sodium fluoride	>24.0	2.2
Parathion 25% WP	>24.0	0.25
Water	—	46.0

[a] Ten termites were placed in each of three beakers containing 1 cc. of powder. The temperatures during the first 3 hours were 73° to 75° F.

[b] Ten termites were placed in each of three 1-inch stender dishes with sufficient slurry to cover approximately half the body surface of the termites, or about 1/16 inch. The same amount of water was used as a check. The stenders were kept covered to prevent evaporation. The temperatures during the first 3 hours were 76° to 78° F.

water under oil, which precludes the possibility of a saturation deficiency at their body surface.

An experiment was made in which the rate of mortality of drywood termite nymphs placed in various powders in 100-ml. beakers was compared with the rate of mortality in the same powders made into a slurry. The slurry was prepared by mixing powder with water until a barely flowable slurry was produced. Thus the termites were in contact with free water, and under such circumstances they evidently imbibe water through their exoskeleton, as indicated by their tendency to swell.

It is evident from table 7 that continuous contact of toxicants in water with the cuticle of the termite provides an effective means of entry of the toxicants into the insect's body. This is also true of the fluorides attached to the silica aerogel particles. Note that without the fluoride coatings these particles (SG 68 and SG 975) prepared as slurries did not result in a complete kill of the termites in 96 hours, whereas in the form of a dry powder they were about as effective as the corresponding coated particles (SG 67 and SS 77).

Abrasion vs. Sorption.—The data presented in table 1 show that silica aerogel powder, the sorptive clays, anhydrone and decolorizing carbon, for example, were more effective than hard, abrasive materials such as carborundum and powdered sand. However, Alumina, a good desiccant, was relatively ineffective. The fact that a mixture of equal parts of Alumina and carborundum (table 1, No. 35) was more effective than either of these materials used separately may indicate that both abrasive and sorptive qualities are desirable. On the other hand, the addition of carborundum to a highly sorptive material, like decolorizing carbon, had the effect of decreasing its effectiveness (compare table 1, Nos. 33 and 36).

Of the four most effective clays, Olancha Clay and Pike's Peak Clay are montmorillonites and Attaclay and Diluex A are attapulgites. However, they are in the "Fuller's earth" category and have in common a high sorptive capacity and very little abrasiveness.

It is of interest to note that Attaclay, one of the effective clays in the present experiments, was also found to be highly effective against the hymenopterous parasite *Aphytis chrysomphali* (Mercet) by Bartlett (1951) and was about as superior to pyrophyllite as in the present experiments.

Table 8.—Loss in weight of lots of 10 live or dead termites dusted with Olancha Clay.

Condition of Termites	Treatment	Per Cent Decrease in Weight During Hours Indicated				
		0–1	1–2	2–3	3–4	0–120
Dead	Dusted; termites undisturbed	0.2	0.2	0.2	0.2	62.2
	Dusted; termites shaken 1 minute	21.3	12.8	7.6	5.8	58.5
	No treatment	0.0	0.0	0.0	0.1	56.3
Alive	Dusted; termites undisturbed	18.6	10.3	6.2	10.3	60.8
	Dusted; termites shaken 1 minute	32.3	13.1	12.1	5.1	64.6

In one test a milliliter of a petroleum spray oil was added to 5 grams of Olancha Clay (table 1, No. 19). This had little effect on the MLP. Despite the large quantity of oil, the dust still had the ability to sorb both oil and water readily. This may be one of the reasons for its success.

The silica aerogels, among which are found the most lethal dusts, are characterized by both extremely small particle size and a higher sorptive capacity for both oil and water than the most sorptive of the common dust diluents, namely, the clays and the diatomaceous earths.

Alexander *et al.* (1944) and Wigglesworth (1945) found that dead insects of species they investigated, when dusted, lost no weight. They concluded that the abrasion occasioned by the movement of the insects on inert dusts removed the relatively water-impermeable lipid surface of the insect epicuticle and resulted in water loss. To check this result on termites, the writers placed 10 termites, with wings clipped, into each of five crucibles. The termites in three of these crucibles had been killed with HCN gas. The live termites in two of the crucibles, and the dead termites in two, were dusted with Olancha Clay by means of a DeVilbiss duster. The live termites in one of the crucibles, and the dead termites in one, were shaken with the dust for 1 minute. The results are shown in table 8. The temperature was 76° F. and the relative humidity was 56%. While the untreated dead termites lost only 0.1% of their weight in 4 hours, the dusted dead termites lost 0.8%. Note that when completely desiccated the termites had lost about 60% of their body weight.

In another test, begun at a temperature of 77° F. and relative humidity of 59%, 20 dead termites dusted with Olancha Clay and left undisturbed lost 15.7% of their body weight in the first 17 hours; those dusted with decolorizing carbon lost 13.4% of their weight; and the untreated check insects lost 1.1% (table 9). As in the experiment depicted in table 8, there was little loss of weight of

Table 9.—Loss of weight of lots of 20 dead termites dusted with Olancha Clay or decolorizing carbon and left undisturbed.

Material	Per Cent Decrease in Weight During Hours Indicated		
	0–3	3–17	17–41
Olancha Clay	0.5	15.2	38.6
Decolorizing carbon	0.5	12.9	42.9
Untreated	0.0	1.1	39.8

the undisturbed dead termites during the first few hours. This indicates that some time was required for undisturbed dust to initiate water loss, but that subsequently water was lost at a progressively greater rate, whereas the loss from the untreated check insects remained practically nil, at least during the first 17 hours. During the next 24 hours it kept pace with that of the dusted insects.

Next the loss in weight was determined for termites that were alive, yet held in such a position that abrasion was not possible. To accomplish this objective 20 weighed live termites, with clipped wings, were attached to cardboard "points" on insect pins, such as are used for mounting small insect specimens. The termites were placed, with dorsum down, on small pieces of chewing gum stuck to the "points." After a short period of vigorous movement of appendages, the termites remained motionless if not disturbed. Ten of the termites were dusted with Olancha Clay using a DeVilbiss duster. The temperature was 75° F. and relative humidity 53%. Among the dusted termites, 50% had died in 5 hours and the loss in weight of the 10 termites at the end of this 5-hour period was 31.3%. Among the untreated termites on "points," all were alive in 4 days and 5 had died in 6 days.

The loss of weight of undisturbed dead termites, and live termites unable to abrade their bodies, indicates that abrasion is not required to initiate desiccation of termites.

As stated previously, Wigglesworth used ammoniacal silver hydroxide solution to reveal the areas from which the surface layer of wax had been removed by abrasion from various species of insects. The polyphenols in the insect cuticle reduce ammoniacal silver hydroxide, causing a black stain.

The writers prepared a 5% ammoniacal silver hydroxide solution by adding 3 ml. of 3N NaOH to 100 ml. of 0.1 N $AgNO_3$, then adding concentrated NH_4OH until the resulting precipitate dissolved. Drywood termite nymphs were used in the experiment because they are white and show up the stained areas to greater advantage than the dark alates. Three groups of termites were placed in the solution. One group had been shaken in a vial with a small amount of Olancha Clay for 1 minute. Another group had been killed with HCN gas and dusted and then allowed to remain undisturbed in the stender dish in which they were dusted for 12 hours. The third group was not dusted and served as the check. All three groups were placed in a sieve and washed under a stream of water, the dust being thereby removed from the treated insects. Each group was allowed to remain in the solution for 2 hours and then immediately photographed. The results of the experiment are shown in figure 4.

The ammoniacal silver hydroxide solution will eventually penetrate the lipid layer of untreated termites and stain the exposed epicuticle, but staining takes place more rapidly if the lipid material is removed. Therefore comparisons of the degree of staining, as affected by different dust treatments, must be made before the solution is able to penetrate the lipid of the untreated-check termites. The latter are shown in figure 4, A. These termites had small, isolated, stained areas, which indicated either that the staining solution had penetrated the thinnest points of the lipid protective barrier or that the latter had been accidentally abraded in a few spots. The dead termites that were dusted and left undisturbed showed large brown-stained areas, later turning black, and some were uniformly stained on the entire ventral surface (fig. 4, B). The termites that were shaken for a minute in the clay were uniformly stained on all body surfaces, indicating a uniform removal of the lipid layer (fig. 4, C).

The above experiment indicated that abrasion is not required for the removal of the outer lipid layer of the drywood termite cuticle. Shaking the termites in the dust greatly aided in the removal of the lipid layer, but it is unlikely that the removal was appreciably augmented by abrasion, for the clay dust used is known to have very little abrasive ability. Presumably adsorption would be augmented by the movement of many particles over a given area as much as abrasion or any other phenomenon occurring at the interface of the epicuticle and the dust particles. As Hurst (1948) has pointed out, the adsorbed lipids are not mobile on the dry dust surfaces, so movement of the dust particles is desirable in order to bring a rapid succession of fresh dust surfaces to the epicuticle.

Despite the evidence for adsorption, it still cannot be inferred that removal of the wax layer takes place in all

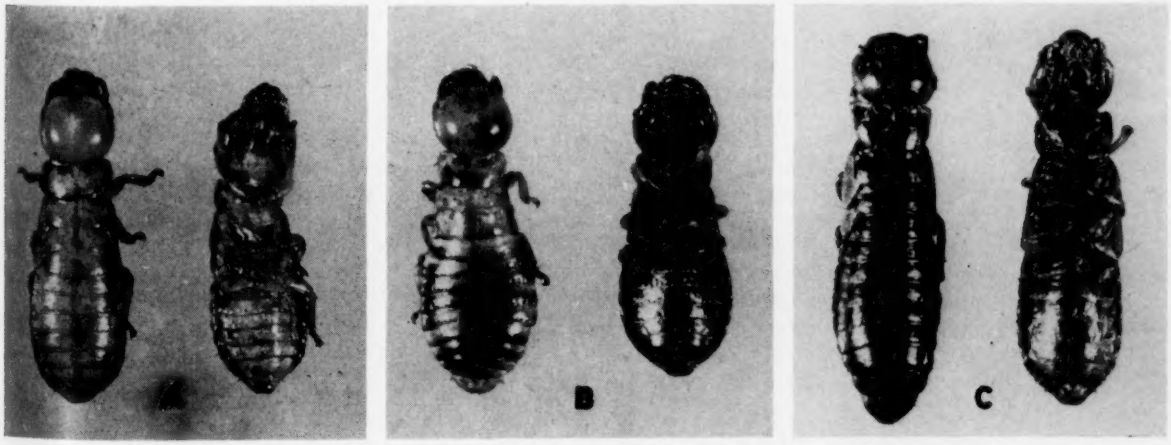

FIG. 4. Discoloration of the cuticle of the termite nymph by immersion in ammoniacal silver hydroxide solution after removal of the surface lipids with sorptive clay dust. A, termites untreated before immersion; B, termites dusted with clay; C, termites shaken with clay dust in a vial.

cases of desiccation resulting from the use of sorptive dusts. The dust particles are generally of a greater diameter than the thickness of the average lipid layer on the insect cuticle (*ca.* 0.25 μ), so it is conceivable that the wax layer might be penetrated by myriads of particles of the dust layer, each of which would act as a minute wick to withdraw water or water vapor. Possibly this phenomenon could not be distinguished from sorption by use of the staining method described above.

EFFECT OF PARTICLE SIZE.—Table 1 shows that the MLP with pyrophyllite (No. 10) was 14.7 hours compared with 3.0 for Olancha Clay (No. 1). Examination of the two materials with a compound microscope revealed that the pyrophyllite particles were on the average much larger than those of the Olancha Clay. When pyrophyllite was ground for 8 hours in a laboratory ball mill it appeared to be much finer and became more effective (No. 11), but still was inferior to the clays. One of the very fine dusts used was carborundum (No. 30), of around 500-mesh size, but it also was less effective than the clays.

Micronized Olancha Clay, with half of its particles below 3.5 μ, and all below 16 μ in diameter, was no more effective than the usual Olancha Clay of commerce, with about half of its particles below 13 μ and all below 100 μ. However, it should be borne in mind that aggregation of particles in the finer dusts may influence results. Other factors being equal, extremely fine particles tend to aggregate more than the larger particles because of the higher ratio of surface to weight.

If a dust possesses a wide range of particle sizes, there will be a sufficient number coming into contact with the insect epicuticle for the desired physical effect, for the small particles will greatly predominate in numbers, even though forming a small percentage of the total bulk of the material. Successively smaller interstices will be filled with successively smaller particles until at the surface of the insect the total area of contact with the dust will be adequate, provided that sufficient dust is applied.

EFFECT OF BULK DENSITY.—In evaluating the data in table 1, Series D, it should be borne in mind that the sorptive dusts were used at a uniform volume (0.5 cc.), but varied greatly in bulk density. The weights of the 0.5 cc. portions of dust, in milligrams, were as follows: SG 67 and 68, 32.8; SG 77, 64.9; Santocel FRC, 25.9; and Olancha Clay, 213. With SG 67, the most effective dust, only 15.4% as much material, by weight, was used as with Olancha Clay, the least effective dust. Since these powders are priced by the pound, bulk density should be taken into consideration, if dust dosages are to be measured by volume.

The comparative value of materials of both low bulk density and a high degree of insecticidal effectiveness was accentuated under actual field conditions of application. For example, in the treatment of the attic of a house for prevention of drywood termites, a comparison was made of the effectiveness of 8 pounds of Olancha Clay, the material currently being used commercially, 3 pounds of SG 77, and 1¼ pounds of SG 67. Three small blocks of wood were suspended in the attic at different distances from the "crawl hole" from which the dusts were applied by means of an electric blower. After the attic was dusted, the blocks of wood were removed and each placed in a 100-ml beaker together with 10 drywood termites. The period required for knockdown and mortality varied greatly with the different degrees of accessibility of the blocks to the point of discharge of the dust. However, the average number of hours for 100% knockdown were 8.5 for Olancha Clay, 1.9 for SG 77 and 1.6 for SG 67. The corresponding periods for 100% mortality were 12.5, 9.2 and 4.8, respectively. These results were typical of two other tests in which the above dusts were compared.

The difference between the three materials was much greater in the field experiment, in which the deposits of dust were light, than in the controlled laboratory experiment depicted in table 1, Series D. This indicates that the relative effectiveness of the more efficient materials increases with decrease in the amount of dust to which the termites are exposed. The more effective silica aerogels are effective against drywood termites when present in films that are barely visible. An additional variable is indicated by the fact that differences between materials became greater with increasing distance of the test blocks from the point of discharge of the dust. Thus in the above experiment when the test blocks were 30 feet from the point of dust discharge and in a direct line with the dust stream, the ratio of knockdown time for Olancha Clay and SG 67 was 3 to 1. However, when the test blocks were about 50 feet from the point of discharge and had to be reached by a circuitous and indirect route, the corresponding ratio of knockdown time of the two materials was 9 to 1. This indicates that the lighter material can more effectively reach the remote extremities of an attic.

DUST VS. SPRAY.—The possibility of applying sorptive diluents in sprays rather than dusts was investigated. Aqueous sprays could not be applied in the attics of finished houses because of the damage that would result from the water penetrating through to the plaster or wall paper. However, the spraying of houses in "rough frame," before the plaster is applied, would be advantageous because of the uniform deposit of diluent that could be obtained. To determine the relative effectiveness of residues left by sprays and dusts, tests were made in which both the spraying and the dusting were done with DeVilbiss equipment. The sprays and dusts were applied to blocks of Douglas fir wood, each with a surface area of 13 square inches.

The results of the experiment are shown in table 10. With comparable deposits, the residues left by the spray were much less effective than those left by the dusts. The diluent particles were dislodged and picked up with greater difficulty as the termites crawled about over the residues. Likewise when one presses a finger against a deposit of dust a large quantity adheres to the skin, but comparatively little material can be picked up this way from a residue left by a spray. Nevertheless, the termites did not

Table 10.—Mortality of drywood termites on sprayed and on dusted blocks of wood.

MATERIAL	MEANS OF APPLICATION	CONCENTRATION (LBS./100 GAL.)	RESIDUE (MG./SQ. IN.)	MLP (HOURS)
Santocel C	Spray	20	2.6	40
Santocel C plus 2% spray oil	Spray	20	7.7	28
Olancha Clay	Spray	20	2.5	170
Santocel C	Dust	—	2.2	1.6
Olancha Clay	Dust	—	3.6	3.5

feed on the wood sprayed with the silica aerogel (Santocel C), but did some feeding on the wood sprayed with Olancha Clay.

The lesser effectiveness of residues applied as sprays is in accord with results obtained by Bartlett (1951) with sprays and dusts of pyrophyllite against hymenopterous parasites on citrus trees. Also in Bartlett's experiments a deposit of dew on dust residues rendered them less effective against the parasites.

The lesser effectiveness of a spray residue must not be construed to imply that the residue, after it is dry, is not intrinsically as lethal as the same quantity of material applied as a dust. It is only its relative unavailability to the insect that causes it to be ineffective. This was proved by placing 10 weighed drywood termite nymphs in dust and 10 in a suspension, of the same dust, which was then allowed to dry on their bodies. Excess dust was removed from the dusted termites by shaking them on a wire screen. However, a film of dust remained on their bodies. The materials used were SG 67, 68, and 77. There was no significant difference in the rate of weight loss for these three materials. The dusted termites lost an average of 35.7% of their body weight in 5 hours. The termites that had been immersed in an aqueous suspension of the powders, then dried, lost an average of 27.2% of their body weight by that time. The ratio of the mortality rates for the two groups was about in the same proportion as the ratio by weight loss.

DEPOSIT OF SORPTIVE DUSTS.—The ability of a dust cloud to deposit material on a solid surface poses no problem when a large quantity can be discharged into a confined space such as in the treatment of attics for prevention of drywood termites. There the dust cloud moves about for a period sufficiently prolonged to enable a large proportion of the particles to impinge on the exposed wood surfaces. In addition, only a relatively light film of dust is required to prevent drywood termite attack. Depositing a sufficient quantity likewise poses no problem when the dust is applied to horizontal surfaces, as in cockroach control. However, there are other control problems in which a heavy film of dust is desirable on vertical surfaces, on ceilings, etc., and where advantage cannot be taken of enclosed spaces. Two of the silica aerogels, SG 67 and SG 77, possess a high positive charge that increases their adherence to solid surfaces. This enables one to apply them to vertical surfaces in a heavy film, by means of a paint brush.

Ordinarily when a dust stream is directed against a vertical surface in open areas, it deposits only a light film, the greater portion floating away. Heavy deposit can be obtained only when using a dust stream of high density and with sufficient velocity to "pile" the dust on the treated surface. On the other hand, the same amount of material applied as a suspension in water or other liquid is deposited much more efficiently. The difficulty exists, however, that the sorptive materials are much less effective when deposited from a water spray, as explained in the preceding section.

The light organic solvents can effectively carry finely divided solids of low density in colloidal solution or suspension and deposit them efficiently when sprayed on vertical surfaces. The solvents volatilize without decreasing the effectiveness of the residue to the same extent as water does. However, practical considerations, primarily relating to mammalian toxicity and/or flammability and sometimes to exorbitant expense, eliminate most of these otherwise effective carriers.

A satisfactory formulation can be prepared, however, by suspending the silica aerogel in Freon with 15% or 30% isopropyl alcohol in an aerosol canister. The alcohol decreases the cost of the preparation and aids in the deposit of the silica. A deposit of any practical quantity can be obtained in both general and localized areas. This makes possible the control of insects that do not succumb on the lighter deposits that are deposited by a dust on vertical surfaces, on ceilings, etc. It also enables one to direct the material to localized areas without contaminating the surrounding areas with dust. This development would greatly extend the range of usefulness of the silica aerogels if it were not for the great expense of using, for example, several aerosol cans for a single treatment for cockroaches that can be effectively controlled with 8 to 12 ounces of the silica in powder form. Unfortunately, the aerosol solution can hold only about 5% by weight of the silica aerogel. Suspensions of these materials in isopropyl alcohol may, of course, also be atomized directly, without recourse to aerosol bombs or canisters. However, residues applied by means of solvents, in aerosols or otherwise, are never as effective as the same quantity of residue when applied as a dust.

THE SPECIAL FEATURES OF THE FLUORINE-IMPREGNATED SILICAS.—The data presented to this point show that the impregnation with fluorides of a silica aerogel, to produce such products as SG 77 and SG 67, has not adversely affected their desiccating ability and at the same time has added to their effectiveness through the toxic action of the fluorine. The advantage of the fluoride "coating" increases with increasing humidity as shown in table 6, for the effectiveness of the fluorides *per se* does not decrease much with increasing humidity and, in the presence of free water, it is actually increased (table 7).

The impregnation of silica aerogels with solid inorganic insecticides is apparently something unique in the field of insecticide chemistry. Insecticide dusts are ordinarily made commercially by preparing "stock dusts" of 25 to 100% of the toxicant and diluting these with the diluent until the required percentage of toxicant is reached. The "stock dust" may be prepared by grinding a solid toxicant with a diluent or by impregnating the diluent with a liquid. In either case the diluent particles in the final dilute dust mixture are surrounded by toxicant particles or by inert diluent particles carrying a charge of usually 25 to 75% of toxicant. As far as the writers are aware only with a few liquid insecticides, such as TEPP and Phosdrin, is the diluent of the final dust mixture impregnated by the toxicant. It appears that currently in no instance is the final dust diluent impregnated with a solid organic or inorganic toxicant except for the silica aerogels that are impregnated with the various fluorides in the process of manufacture.

One instance was found in the U. S. Patent Office literature of a mineral being coated with a fluoride. Peddrick (1938) recognized the ability of pyrophyllite to kill insects by desiccation. By treating pyrophyllite, as well as other minerals such as bauxite, alunite, and topaz, all of which contain alumina, with a liquid or gaseous reagent contain-

Table 11.—The relative effectiveness against two species of insects of silica aerogels with and without fluorides added by impregnation and by mechanical mixture.[a]

Material	Period for 100% Mortality (Hours)	
	Termites	Cockroaches
SG 68	1.7	5.2
SG 67	1.0	1.6
SG 68+4.7% ammonium silicofluoride	1.7	4.2
Ammonium silicofluoride	4.2	2.0
SG 975	1.6	5.7
SG 77	0.9	1.7
SG 975+3.85% magnesium silicofluoride	1.5	2.8
Magnesium silicofluoride	4.7	2.5
Sodium fluoride	4.7	2.9
L.S.D. at 5% level	0.45	1.26

[a] The test insects were alate drywood termites and adult male German cockroaches. All the fluoride compounds had been passed through a 400-mesh screen. Temperatures and relative humidities were: for termites, 76 to 78 F., 59 to 60% R.H.; for cockroaches, 72 to 77 F., 60 to 62% R.H.

ing a fluorine radical, Peddrick was able to etch the surfaces of the mineral particles so that each contained a "partial coating" of aluminum fluoride (AlF_3). The ability of the finely divided minerals to cause insects to lose water was thereby enhanced and, in addition, advantage was taken of the toxic effect of the fluorine. However, Peddrick's process was limited to certain minerals containing alumina and which can be shown to have a comparatively low order of insecticidal effectiveness. According to Peddrick's data, the insecticidal effectiveness of pyrophyllite against Japanese beetles, *Popillia japonica* Newm., was increased only about two-fold by the aluminum fluoride coating.

The advantage of impregnation, compared with a mechanical mixture of silica and fluoride particles, was demonstrated in an experiment in which the fluorides added to SG 68 and SG 975 to produce SG 67 and SG 77, respectively, were ground by means of a Waring Blender and the portions of the powders passing through a 400-mesh screen were used. Ammonium silicofluoride was mixed with SG 68 to form a 4.7% concentration and magnesium silicofluoride was mixed with SG 975 to form a 3.85% concentration.

Alate drywood termites were placed in jars with bottoms of an area of 3 sq. in. and containing 1 cc. of powder. Adult male German cockroaches were placed in 1-quart jars containing 200 mg. of powder. The periods required for 100% mortality were recorded and the results are shown in table 11.

With termites, impregnated silica aerogels were, on the average, 1.7 times as effective as the unimpregnated materials, 1.7 times as effective as the silica-fluoride mixtures, and 4.7 times as effective as the fluorides themselves. With cockroaches, the fluoride-impregnated silica aerogels were 3.3 times as effective as the unimpregnated materials, 2.2 times as effective as the silica-fluoride mechanical mixtures, and 2.2 times as effective as the fluorides themselves.

The writers believe that the silica aerogel particles, because of their ability to remove or disrupt the lipid layer, act as effective vehicles for the entry of water-soluble inorganic toxicants to hydrophyllic tissue. The greatest advantage can be taken of this phenomenon if this vehicle is impregnated by the toxicant, so that each particle contacting the cuticle has its charge of toxicant. Thus there exists a layer of toxicant in contact with the cuticle, instead of a mixture of toxicant and diluent, with the latter predominating, such as results from the usual diluent-toxicant mixtures.

The silica aerogel particles, though extremely small, are porous. The total surface area of a gram of material is enormous. For SG 975 and SG 68 it is 340 and 300 square meters, respectively. When these materials are impregnated with 3.85% and 4.7%, respectively, of the fluoride, it is believed that the latter exists in something less than a continuous monomolecular layer. Therefore it must be assumed that much of the fluoride added to these particles, and perhaps the major part, is inside the particle. However, being water-soluble, it may be extracted upon reaching the procuticle of the insect, which contains a "fairly high water content" (Roeder 1953). At any rate, the insecticidal efficiency of the impregnated dusts, compared with the conventionally prepared dusts, can be convincingly demonstrated.

The lipid material on the epicuticle of the cockroach is relatively polar in nature and therefore fluorides have considerable contact effectiveness, as shown in table 11. This is true if ingestion is completely eliminated as a possible contributing factor. In one experiment 10 cockroaches were laid on their backs on each of a number of 3×5 cards by passing strips of Scotch tape over their heads. This prevented preening or accidental contamination of the mouthparts. Also it prevented the cockroaches from moving about. The various powders listed in table 11 were poured onto the bodies of these cockroaches, a different powder on each group of 10 insects. The excess was then tapped off. These immotile insects died in only 66% of the time required for the motile insects crawling about in the residues, shown in table 11, apparently because a greater quantity of the fluoride could be brought in contact with the epicuticle. Motile cockroaches do not "pick up" the fluorides, even though they may be extremely finely divided, as well as they do the silica dusts. On the other hand, the cockroaches treated with the silica dusts required 20% longer to die in the stationary position than they did when crawling over the dust residues.

It is another indication of the high degree of insecticidal efficiency of the impregnated silicas, SG 68 and SG 77, containing only 4.7% and 3.85%, respectively, of the fluorides, that they should kill cockroaches in only 46% of the period required by 100% concentrations of the fluorides themselves. With the termites, having a less polar lipoid protective layer, the difference in favor of the impregnated silicas was much greater; they killed in only 21% of the period required by the fluorides. The action of the diluents in bringing the fluorides into contact with hydrophyllic tissue was of greater importance in the case of the termites.

Sodium fluoride is the old-fashioned "cockroach powder" and is again being used in increasing quantities by pest control operators in the face of increasing evidence of cockroach resistance to the chlorinated hydrocarbons and organophosphorous compounds. The average period required for SG 77 and SG 67 to give a 100% kill of cock-

roaches was only 57% as long as that required by a 100% sodium fluoride powder.

DESICCANTS OTHER THAN INERT DUSTS.—A number of insecticides and other compounds can cause insects to lose water by means not always involving the removal of the lipoid protective layer. They may be effective as solutions or emulsions, as well as in dust form. For example, parathion can cause a severe desiccation and shrivelling of mites and termites. On the other hand, Chlorobenzilate and Aramite leave mites with such a natural appearance and posture, after they die, that these acaricides have been used for the preparation of mites for photography. The same may be said for pentachlorophenol and sodium pentachlorophenate when used against termites (Ebeling & Pence 1957). Interesting observations can be made when insects treated with certain substances known to cause desiccation are placed under oil in order to observe the presence or absence of water droplets on the cuticle.

Wigglesworth (1942) found that when refined petroleum oil was placed on a small insect and a coverslip was placed over it, minute droplets of water could be seen to issue from the surface of the cuticle. These appeared in about 30 minutes; at the end of 2 hours the droplets were numerous. They appeared even on isolated portions of appendages of the insects. Wigglesworth believed that this phenomenon demonstrated that oils dissolve or permeate the lipoid film on the surface of the cuticle and reach the water in its deeper layers. He believed that the oil may dissolve long-chain polar substances from the cuticle and that these substances in the oil-water interface may result in water being drawn from the insect. The senior writer accomplished the same result by adding 1% glyceryl monooleate to medium summer spray oil,[3] for this compound greatly decreases the interfacial tension oil/water. Tests with a du Nouy tensiometer showed that the interfacial tension, at 72° F. of water and a light medium spray oil, could be decreased from 31.4 dynes/cm. to 1.5 dynes/cm. by the addition of 1% glyceryl monooleate to the oil. Entirely aside from the effect of decreasing interfacial tension oil/water is the possibility of the glyceryl monooleate aiding in dissolving or disrupting the lipids on the insect epicuticle. Striking examples of rapid desiccation were obtained by Wigglesworth (1945) by painting similar compounds (glyceryl monolaurate and diglycol oleate) on the bodies of the nymphs of *Rhodnius*, the pupae of *Pieris*, and the larvae of *Nematus* and *Tenebrio*.

Beament (1945) showed that the wax layer on the insect epicuticle can be readily penetrated by oil and oil-soluble materials. These upset the orientation of the lowest monolayer of wax, the one he believed to be the most effective as a barrier to water loss. This brings about the irreversible changes in permeability reported by Hurst (1943). Beament (1945) believed that the solvent most effective in withdrawing water from an insect is the one with the most suitable balance of hydrophilic and lipophilic characteristics.

The spray oil by itself had little effect in drawing water from termites. Therefore, it was interesting to note that certain compounds known for their desiccating effect, such as parathion and sodium fluoride (see Ebeling & Pence 1957) caused copious quantities of water to accumulate on the bodies of treated termites when they were immersed in oil 30 minutes after treatment. When termites were dusted with anhydrous magnesium perchlorate, which soon deliquesced, and were then placed under oil, water was withdrawn in large quantities within 3 hours (fig. 5). In 24 hours it formed large pools, in which the termites lay. When termites were placed in aqueous solutions or suspensions of the above substances, then placed in oil, the water issued from their bodies the same as when they were dusted.

FIG. 5.—Water that issued from the cuticle of a drywood termite that had been dusted with anhydrous magnesium perchlorate, allowed to remain for 30 minutes, and then had been immersed in spray oil for 3 hours

On the other hand, when termites were placed under oil after having been dusted with inert diluents, little or no water was withdrawn from their bodies. However, if the termites were vigorously shaken in the dust for a moment, water issued from their bodies in copious amounts when they were washed and dried and then placed under oil. Olancha Clay, being about six times heavier than SG 67, had a greater effect than the latter. Termites or German cockroaches shaken 10 times in vials with Olancha Clay and held under oil suffered about as rapid a loss of water, within a few hours, as is indicated in figure 5. It is interesting, however, that insects merely dusted with the sorptive dusts, without violent agitation, lose little or no water when placed under oil, whereas insects treated in a similar way with other substances, such as parathion, sodium fluoride, and anhydrous magnesium perchlorate, do lose water in a conspicuous manner, as shown in figure 5. Yet weighing experiments show that the sorptive dusts are the more effective in causing loss of water from insects left in the open air.

The fluorides ordinarily desiccate termites much less rapidly than silica aerogels, as may be seen from the data presented in table 5, and which has been substantiated by other experiments with ammonium silicofluoride, magnesium silicofluoride and sodium fluoride powders ground to 400-mesh and compared with silica aerogel dusts on unconfined surfaces in a ventilated room.

In one experiment drywood termite nymphs were weighed and were then covered with powder and the excess was removed by shaking the termites on a screen.

[3] Naphthenic petroleum oil, 50% of which distills at 650° F. (760 mm. Hg).

The termites were then confined in celluloid vials 2 inches high and ½ inch in diameter during the desiccation and weighings. The rate of desiccation was greatly retarded, for in a narrow vial evaporation cannot readily take place. Under such conditions the difference in the rate of water loss of insects treated with silica aerogels and those treated with fluoride powders was decreased. The temperatures during this experiment ranged from 76° to 78° F. and the relative humidities ranged from 57 to 60%.

In 10 hours there was an average loss of body weight, caused by desiccation, of 35.7% for SG 67, 68 and 77, an average of 25.9% for ammonium silicofluoride, magnesium silicofluoride and sodium fluoride, and 17.8% for 25% parathion emulsion. In the same period the loss of body weight of the untreated termites was 5.7%.

Sodium pentachlorophenate is one of those insecticides that ordinarily has no appreciable effect on water loss of termites (Ebeling & Pence 1957). Nevertheless, this compound causes water to issue from the bodies of termites immersed in oil after treatment. However, this may be explained by the ability of sodium pentachlorophenate to decrease the interfacial tension oil/water which, as previously explained, has the effect of causing oil to draw water from the body of an insect. The other compounds tested, however, had no appreciable effect on interfacial tension oil/water, and no explanation can be offered at this time as to the mechanism by which they cause water loss in insects, either exposed or immersed in oil. Obviously they decrease the ability of the epicuticle to retard loss of water or water vapor.

If termites are immersed in oil to which a surface-active solute has been added, and the oil is then drained from the surface of their bodies by placing the insects on several layers of paper towelling, these termites will lose water more rapidly than those treated in the same manner, but with straight oil. Twenty termites so treated with the previously mentioned oil and 20 termites treated with the same oil plus 1% glyceryl monooleate were weighed 1, 2, 8.5 and 14 hours after treatment. The weight of the film of oil remaining on the two groups of 20 termites immediately after treatment was 14 mg. for those treated with straight oil (A) and 13 mg. for those treated with oil plus glyceryl monooleate (B). In 1 hour A and B each lost 12 mg. in body weight. Between 1 and 2 hours after treatment, A lost 2 mg. and B lost 8 mg. Between 2 and 8.5 hours A lost 22 mg. and B lost 42 mg. Between 8.5 and 14 hours A lost 14 mg. and B lost 15 mg. Thus between 1 and 8.5 hours B lost slightly over twice as much weight as A, demonstrating that a solute that decreases interfacial tension oil/water will accentuate the desiccation of an insect whether the insect remains in the oil or whether the oil is removed, exposing the insect to the air.

Ebeling (1950) immersed potato tuber moth larvae (*Gnorimoschema operculella* Zell.) in light medium spray oil with and without 1% concentrations of various surface-active solutes and immediately absorbed the excess oil from their bodies by placing them on paper towelling. The MLP's (in minutes) obtained with the four oils were as follows: oil alone, 275 minutes; oil with a glyceryl monooleate, 93 minutes; oil with glyceryl dioleate, 111 minutes; and oil with glyceryl trioleate (glyceryl oleate), 120 minutes (least significant difference = 45.5 minutes). The number of OH radicals possessed by the solutes are 2 for glyceryl monooleate, 1 for glyceryl dioleate, and none for glyceryl oleate, indicating the approximate ratio of their polarity. The larvae treated with oil plus solute were conspicuously desiccated before those treated with oil alone had suffered any appreciable loss in turgidity.

DISCUSSION.—Evaporation, a function of surface area, increases as a threat to the existence of terrestrial organisms with decrease in their size and consequently increase of the ratio of surface to volume. Insects have on their epicuticle a very thin layer of lipid, mainly wax, which serves admirably as a protection against desiccation, provided that it remains intact. However, it is an intriguing thought that this precariously thin film of lipoid material, variously estimated to be 0.1 to 0.4 μ thick, protects the insect from desiccation and death that can take place more rapidly than that resulting from the most toxic of the modern "superinsecticides."

Most investigators working with desiccating dusts have assumed that the desiccation of the treated insects depended on the removal of their waxy protective layer by abrasion. However, a few produced irrefutable evidence that dusts can adsorb this lipid material: Wigglesworth (1945) by using dead insects and sorptive dusts (cockroaches); Hurst (1948) and Jones (1955) with water-suspension methods (*Tenebrio* larvae and bees); Helvey (1952) by means of electron microscopy (*Epilachna* larvae); and the present writers with staining methods (*Kalotermes* nymphs and *Oryzaephilus* adults). The preponderance of evidence for adsorption, however, is that, with most insects, the least abrasive and most sorptive dusts have been the most effective.

Once having established that removal or disruption of insect wax can be accomplished on a wide variety of insects and mites by adsorption, the control of pests by dust desiccation assumes a new significance. While desiccation by abrasion can be effective primarily against such insects as the granary weevils, whose habits and environment make them amenable to this type of action, desiccation by adsorption is not so severely limited in its scope. Experiments to date have already showed that many insects and mites of widely different types and habits are amenable to control by dust desiccation.

By fortuitous circumstance, silica aerogels impregnated with water-soluble inorganic fluorides already existing as an item of commerce in the paint and other industries have added to dust desiccation the factor of toxication. Once these fluorides have penetrated the epicuticle, most commonly about 1 μ in thickness, the fairly high water content of the procuticle or chitinous cuticle apparently changes the water-solubility of the fluorides, previously a liability, into a distinct advantage, and a remarkably high degree of contact toxicity is the result. Thus the addition of small percentages of inorganic fluorides has further increased the potentialities of the desiccating dusts in pest control by greatly increasing their insecticidal efficiency and by making their effectiveness largely independent of variation in humidity, thus increasing their versatility.

Three important limitations in the use of most insecticides would be avoided by the use of sorptive dusts or powders to combat those insects that are amenable to control by dust desiccation (1) hazard to humans and other

warm-blooded animals, (2) loss of effectiveness through chemical decomposition, and (3) loss of effectiveness through insect resistance. With regard to the latter, it is believed that since the sorptive dusts kill either by physical means alone or by a combination of physical and chemical means, depending on their composition, insects may not develop resistance to them.

The current return to the use of sodium fluoride for control of resistant cockroaches indicates that the contribution to the effectiveness of certain silica aerogels that is attributable to fluoride impregnation is not likely to be diminished by the development of resistance, or at least not for many years.

The prevention of infestation of wood structures by drywood termites, the elimination of colonies of bees and wasps from buildings, the control of cockroaches, fleas on dogs and cats, fungus flies in mushroom houses, and two-spotted spider mites in rose ranges, by means of silica aerogel dusts, have already showed promise in field experiments made at this Experiment Station and in limited commercial use. Further investigation may reveal the potentialities of this method of control for other pests.

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Lethality of Inert Dust Materials to *Kalotermes minor* Hagen and Their Role as Preventives in Structural Pest Control[1]

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ABSTRACT

Samples of 162 insecticide diluents and other finely divided inert materials were first screened in a pilot test in which drywood termites were placed in 1 cc. of the material in 100-ml. beakers, then in a test in which they were confined with blocks of wood that had been dusted at an air velocity of 1,400 feet per minute in a specially constructed wind tunnel. In the latter tests, the four outstanding groups of materials, in descending order of effectiveness, were silica gels, aerogels, or precipitates > montmorillonite clays = attapulgite clays > diatomites.

In tests made in attics of residences, blocks of wood were placed in a number of locations representing different distances and degrees of accessibility from the point of discharge of the dust. After the dust was applied the wood blocks were taken to the laboratory and termites were confined with them. These tests revealed a property of the sorptive dusts not evaluated in the pilot or wind-tunnel tests, namely, their bulk density. The lighter materials were more uniformly distributed throughout the attic and their superiority to the heavier materials increased with increasing distances from the point of discharge.

In the course of investigation of toxicity of insecticide formulations, applied on wood, to the drywood termite, *Kalotermes minor* Hagen, it was noted that after 17 months of storage in an average attic, only dust formulations were lethal. It was further noted that lethality was proportional to the amount of dust on the wood and not to the toxicants which had been used. Since the only component of the dust formulations which remained in many cases was the diluent, a comprehensive screening of as many dust diluents and finely powdered industrial materials as possible was made. Samples of 162 products, encompassing the majority of the known range of insecticide diluents, as well as many finely divided powders used for a wide variety of purposes in industry, were obtained from 47 manufacturers and suppliers and chemical concerns throughout the country.

A discussion of the physical and biological factors that pertain to the lethal action of inert dusts, as well as the literature on this subject, is contained in a companion paper by Ebeling & Wagner (pp. 190 to 207). It is pertinent to a proper understanding of the present paper, however, to state that the finely divided inert materials, applied as dusts in the present investigation, kill insects by removing or disrupting the lipoid protective layer on the epicuticle, causing them to die from rapid desiccation. The effectiveness of the dusts against insects is related to their ability to sorb lipid material and the particle size.

The purpose of the present paper was to screen as large a number as possible of inert dust diluents, or other finely divided substances that have been used for various industrial purposes, in order to find promising materials for application to exposed wood surfaces of uninfested newly constructed residences or those that have been attacked and recently successfully treated for control of drywood termites. Since dusts could be expected to be effective only for the prevention of new infestations, termites already infesting a building had to be eradicated by some other means before the preventive dusts were applied.

MATERIALS AND METHODS.—All dusts received were used in a pilot test to determine inherent lethality to *K. minor*. In this test approximately 1 cc. of each material was placed in each of three 100-ml. beakers and 10 late nymphal instar termites were placed directly into the dust layer thus formed. The beakers were held in a room at moderate temperature (60° to 80° F.) and high humidity (60% to 100%) where the number of hours required for 100% kill was recorded up to a maximum of 9 hours. Average number of hours for 100% kill for each material is shown in table 1 in the pilot beaker test column. Further testing was limited to those materials which caused 100% mortality in 5 hours or less.

To simulate field conditions and take into consideration not only inherent lethality but depositability of each material, a small wind tunnel was constructed in which wood blocks could be uniformly dusted. The wind tunnel is illustrated in figure 1. The tunnel was fabricated with ventilation ducting 4 inches in diameter, using two 90° elbows soldered together for each end and 30″ straight lengths for the sides. Both ends of the tunnel were wrapped with masking tape to seal all joints air tight. An impeller was made from a six-bladed electric motor cooling fan which was cut down to $3\frac{3}{4}$″ diameter and balanced. The impeller was mounted on a $\frac{1}{4}$″ steel shaft which was supported within the tunnel by a ball bearing mounted to three supports at 120° from each other which were bolted through the tunnel wall. The shaft was run through a $\frac{3}{8}$″ hole in the tunnel end and supported outside by a second ball bearing. A $\frac{1}{2}$″ diameter drive pulley was secured to the outer end of the impeller shaft to complete the drive assembly. Immediately before the impeller, a funnel was fitted into the top of the tunnel through a rubber stopper and discharged its dust contents gradually, directly into the air stream. Since the impeller imparts a rotary flow to the air, a stabilizing vane assembly consisting of thin aluminum plates 2″ in length in a 1″ square honeycomb configuration was placed within the tunnel 2″ in front of the dusting chamber. A swinging blade type of velocity gauge was installed directly before the dusting chamber. The dusting chamber consisted of a window 6 inches in length and one-half the circumference of the tube in width covered by a removable lucite chamber cover which sealed to the tunnel with sponge rubber weather-stripping. A test block mounting crank into which blocks of wood were snapped was fitted horizontally through the tunnel centered in the test chamber. These blocks were of planed fir wood with dimensions of

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Table 1.—Period required for 100% mortality of drywood termites placed in beakers with 1 cc. of diluent.

MATERIAL	HOURS
BOTANICALS	
almond shell flour	—[a]
apricot pit flour	—
apricot flour	—
fine apricot pit charcoal	—
olive flour	—
peach flour	—
pecan flour	—
spray soy A	—
spruce bark flour	—
walnut shell flour WF-71 (1)[b]	—
Agicide DB-4 dust conc. (1)	—
dust of Agicide (1)	—
soybean hull flour (36)	—
Orgafil (36)	—
corn gluten (36)	—
soy flour I (36)	—
soy flour F (36)	—
starch (36)	—
corn steep liquor (36)	—
Solka Floc BW-40 (6)	—
soy bean mill feed (5)	—
ELEMENTAL MINERALS	
sulfur, electric (37)	—
OXIDES OF SILICON	
Diatomites:	
*Celite 209 (25)	3
*Dicalite IG-1 (8)	3
*Dicalite IG-3 (8)	3.25
Dicalite IG-5 (8)	8
*#680 Multicel (38)	4.5
*MN-41 Celatom (41)	4
*Diacron C (22)	3.75
*#1451 diatomaceous earth (43)	3.5
*anhydrite filler (30)	4.0
*Diatomite (30)	3.5
Tripolites:	
silica #1240	5.5
Cream Tripoli, once ground (4)	8
Cream Tripoli, twice ground (4)	8
Cream Tripoli, airfloat (4)	7.5
*Rose Tripoli, once ground (4)	4.5
*Rose Tripoli, twice ground (4)	4
*Rose Tripoli, airfloat (4)	3.75
Dustex Micro Silica (38)	6.5
#39 silica (41)	7
silica #410 (7)	7.5
silica EF (7)	8
silica EEF (7)	3.5
*silica, 400 mesh (42)	2.7
silica DCS (7)	—
#22 silica (43)	5.75
Synthetics:	
*Type A-1 aerogel (15)	2.25
*Type AO-1 aerogel (15)	2.7
*Type AO-2 aerogel (15)	2.05
*Hi-Sil 233 (13)	2.25
*Hi-Sil X-303 (13)	2.0
*Santocel C (29)	3.5
*Santocel 54 (29)	5.0
*SG 72 (14)	2.75
*SG 971 (14)	2.75
*SG 976 (14)	3.0
*SG 68 (14)	3.0
*SG 67 (14)	2.75
*SG 77 (14)	2.50
*SG 74 (14)	3.0
*SG 97 (14)	2.5
*SG 78 (14)	2.5
*Valron estersil (17)	4.75
OXIDES OF MAGNESIUM	
*Magnesite, caustic burner (43)	4.05
OXIDES OF IRON	
Red Iron oxide (43)	—
CARBONATES	
Calcium lime:	
pulverized lime (27)	7.0
pulverized lime #200 (27)	7.5
Mississippi diluent (untreated) (28)	—
Mississippi diluent (treated) (28)	—
Guado FF aero lime (3)	—
Hydrated calcium limes:	
Acme hydrated lime	7.0
hydrated lime (31)	
S & D hydrated lime	(27) 5.5
*Sweet Arrow lime (27)	4.0
Magnesium lime:	
Stonelite (31)	7.5
Calcite:	
Enamel white calcite (23)	7.5
B. I. #1 white (39)	8
Whiting 400 mesh (42)	8
Calcene NC (13)	7
#1887 Cal-dust A (43)	—
SULFATES	
Gypsum	
Blue Diamond gypsum	—
*calcined gypsum (9)	4
Barytes:	
Barytes (23)	—
#89 Barytes (43)	—
SILICATES	
Mica:	
#1473 Mica (43)	—
vermiculite (43)	—
ground sericite mineral	4.5
Talcs	
blue talc (46)	—
Sierra talc (34)	5.5
Huntley talc	6.0
400-mesh talc (42)	5.0
#5 insecticide talc	—
Marten white	8.0
Marten white 325-mesh	—
Sierra IR white talc (34)	7.0
*Sierra Mistron Vapor (34)	5.0
*Mineral Mix (23)	5.0
Alabama talc (20)	5.5
Georgia talc #901 (20)	6.0
Texas talc (20)	6.0
*Loomite talc (24)	5.0
GJ grey talc (38)	8.0
*Nytal 200 (40)	4.5
*Velvatone talc (9)	4.5
Emtal 23A (18)	—
Emtal 42A (18)	7.5
Cahutta #2 talc (12)	8.0
*Asbestol regular (10)	4.0
*#5 Canadian talc (43)	7.5
#367 Georgia talc (43)	5.5
*Soapstone (30)	4.5
*Velvalite	4.5
*#1718 Fibrous Mineral Filler (43)	4.5
*#2952 Clatal (43)	3.0
SYNTHETIC CALCIUM SILICATES	
*Silene EF (13)	3.75
*Microcel E (25)	4.0
Pyrophyllites:	
*Pyrax A (40)	4.0
*Pyrax B (40)	5.0
Pyrax AEB (40)	6.0
Phyllite	6.0
#2 Phyllite	7.5
*Pyrol	4.5
Pyrolite	8.0
*Super Pyrolite	4.0
*Amcolite #1	3.0
insecticide grade pyrophyllite (21)	—
#29 pyrophyllite	6.28
Nu Clay	5.5
X-5 white talc	3.75
Aluminum silicates:	
Pelbon (41)	—
Montmorillonite clays:	
Filtrol X-415	5.5
Wyoming Bentonite	—
*Pike's Peak clay #9S-66 (19)	4.5
*Pike's Peak Clay #9T-66 (19)	4.75
*Olancha Clay (34)	3.0
*Olancha Clay micronized (34)	2.25
Bentonite SFV (2)	5.5
#890 Volclay Bentonite (43)	8.0
*#140 Bleaching Clay (23)	5.0
*Alvin J. Cox's Earth	3.0
Kaolinite clays:	
*KT Airfloat clay	5.0
Inert "C"	6.5
*Barden clay	5.0
Type 41, Kaolin	7.0
Cherokee clay	6.5
Type IG Kaolin	8.0
GX #22 Kaolin (33)	6.0
*"L" China clay (38)	4.5
*Kaolloid clay (39)	4.5
B-6 clay (23)	6.5
*B-5-6-2 clay (23)	4.5
*#1431 clay (43)	4.0
Attapulgite clays:	
*Attaclay (44)	2.75
*Diluex A (45)	2.25
*Hi-Dry (calcined)	3.25
VOLCANIC MATERIALS	
Frianite DS (8)	6.0
Frianite M3X (8)	5.5
*#245 Volcanic Ash (43)	5.0
*#181 Feldspar (43)	4.5
INDETERMINATE	
*#188 slate flour (43)	4.0
*#2133 slate flour (43)	3.5

[a] The data are given only for those materials that resulted in a 100% mortality in 8 hours or less.

[b] Numbers in parenthesis refer to those given in table 1 and indicate which firm contributed the respective diluent.

Table 1.—(*Continued*)

$2\frac{1}{4} \times 1\frac{1}{2} \times \frac{3}{4}$ inches. A $\frac{1}{2}$" mesh hardware cloth safety screen was soldered in the tunnel behind the test chamber to prevent any blocks which might be torn loose from their mounting from being drawn into the impeller.

The air velocity was controlled by varying the impeller speed. This was accomplished with a variable speed electric motor and transmission unit (Master Speedranger Model V-21) which supplied shaft speeds from 180 r.p.m. to 2,750 r.p.m. By using a 5-inch drive pulley on this unit coupled to the $\frac{1}{2}$" impeller drive pulley by a nylon belt, an impeller speed range of 1,800 r.p.m. to 27,500 r.p.m. was obtained. By placing an anemometer (Taylor No. 3132) in the test chamber and varying the drive speed, a blade velocity gauge was calibrated. Although the anemometer retarded the air velocity relative to the drive speed, the deflection of the velocity gauge blade was controlled by the air velocity. In operation, the velocity gauge was used only in adjusting air velocity preparatory to dusting and was locked out of the air stream during dustings.

To determine the optimum dusting velocity within this unit, a microscope slide was taped to the test block mounting as a base for the 1" by 3" wood veneer (weldwood flexwood) strips. The strips were preweighed and placed in groups of three for dusting at various velocities; Attaclay was selected as a standardizing diluent, since it is one of the best known, most widely distributed diluents showing inherent lethality. Five cubic centimeters of this diluent was added to the funnel hopper and a veneer strip was placed on the mount in the dusting chamber. The drive motor was turned on at a pre-set speed and was run for 3 minutes, turned off, and the test strip was removed. The second and third strips of each series were treated in the same manner with the dust remaining in the tunnel from the original 5 cc. After each series, the tunnel was blown clean by turning on the drive motor with the lucite chamber cover inverted into the tunnel and used to deflect the air streams out and up. Compressed air was used as a supplementary cleaning medium and was blown through the tunnel at all openings until no dust was evident in the exhausted air. The veneer strips were then reweighed to determine deposit per unit area. This procedure was followed at 600 ft. per minute and increments of 200 ft./min. velocity up to approximately 2500 ft./min. From the data thus obtained an optimum dusting velocity of between 1300 and 1500 ft./min. (15 to 17 m.p.h.) was determined which gave the heaviest even dusting. Below this velocity the dust aggregates were not broken down and, above, dust built up in mound formations. A mean velocity of 1400 feet per minute was used in all screening dustings which followed.

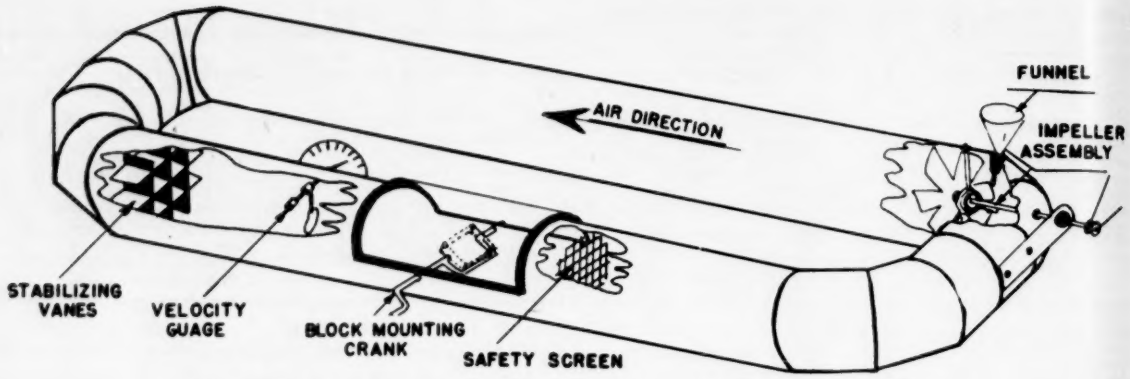

FIG. 1.—Wind tunnel used for applying dust to wood blocks at a known velocity.

To test the effectiveness of each lethal dust as it would be used in the field, Douglas fir wood blocks 2.25″×1.5″ ×0.75″ were snapped into the test block mounting and dusted. By using the 5 cc. volume of dust previously indicated, it was found that the dust deposit was heavier than that normally achieved in the field. For this reason the volume for all screening was decreased to 3 cc. for the first block with an additional 1.5 cc. before each, at the second and third replicate blocks, to make up for dust lost through leakage from the tunnel and deposited on the previous block. This dosage gave a deposit commonly surpassed in the field. Each material killing in 5 hours or less in the pilot test was dusted in this manner for 30 seconds on each block of a 3-block replicate group. The dusted blocks were placed directly into 100-ml. beakers which were then put into the testing room described above for a minimum of 12 hours, in order to come to moisture equilibrium with the atmosphere. After this period, 10 late instar nymphal termites were placed in each beaker and the time to 100% immobility and 100% kill was recorded.

The use of alate (swarmer) termites was impossible in many of these tests as they were available for only a limited period during the summer months. An experiment was conducted with both alate and nymphal termites to compare their susceptibility to diluent-caused mortality during the period when alates were available. The results of this test proved that alates were slightly more susceptible (1% level of significance) than the nymphs.

Field tests in attics of residences, with the materials shown most promising in the previously described tests, have been made to determine which materials afford the greatest degree of protection from drywood termites. Dustings have been made with various types of dusters ranging from hand-operated Root dusters to electric "Tornado" dusters[2] (commercial vacuum cleaner motors fitted with a dust hopper and discharge nozzle). Due to the density of dust cloud formed in the confined areas of attics, movement of the operator to various parts of the attic is very difficult and hazardous but is required with the hand-operated equipment. The electric "Tornado" duster used in the tests achieves an excellent dispersion of dust from the crawl hole opening and does not require the operator to actually move through most attics during dustings.

RESULTS.—Results of the pilot tests of all materials are given in table 1. All materials listed in table 1 that were later tested in the wind tunnel are marked with an asterisk. Results of the wind tunnel tests are shown in table 2 with the exception of those materials that required more than 8 hours to cause 100% immobility. In both tables the data for different materials are not necessarily directly comparable, for the tests extended over a considerable period of time. Despite an effort to control temperature and humidity, these varied over a considerable range on different days, as previously indicated. However, the temperatures were lower and the humidities were higher than those which would ordinarily be encountered in attics in southern California during the periods of termite swarming. Therefore the data shown in tables 1 and 2 may be considered conservative.

Wind tunnel tests showed four general groups of promising materials. They ranked, in the order of effectiveness, cilica gels, aerogels or precipitates > montmorillonite clays = attapulgite clays > diatomites. Within each group considerable variation in lethality was found which appeared to be dependent on three major factors: oil-sorption capacity, water-sorption capacity, and particle size. In general the greater the sorptive capacity and smaller the particle size the more lethal the material was.

Since the aim of this research was to discover a practical material for the treatment of structures, certain other characteristics were required. These were low cost, ability to float in the air stream to the most remote corners of the dusted attics, and effectiveness at very low deposit levels. The clay and diatomite materials are quite effective in preventing drywood termite infestations, but due to their relatively high density will not carry and deposit sufficiently well with dusting equipment easily transported into home attic areas and easily carried by a pest control operator. The dense dust clouds which are confined by the structure make movement of the operator nearly impossible during the dusting and make the carrying capacity of a dust of primary importance.

The silica gels, aerogels and precipitates combine all of the desirable characteristics for dusting, as well as being the most lethal of all materials tested. Among those tested the "SG" series proved in field practice to be the most effective.

The relative potentialities of some of the silica gels and

[2] Courtesy of Black J. Chemical Company, Santa Monica, California.

aerogels are not completely depicted in table 2. Two of the materials—a silica gel (SG 77) and a silica aerogel (SG 67)—were impregnated with 3.85% magnesium silicofluoride and 4.7% ammonium silicofluoride, respectively, which caused them to take on a strong positive charge when submitted to friction. They tended to adhere to the sides of the wind tunnel with a high percentage not reaching the wood blocks. This is especially true of SG 67. Also since the materials were used at equal volumes they differed greatly in weight, the unit on which the cost of materials is based. From the data shown in table 2, SG 67 and Olancha Clay do not appear to differ greatly in insecticidal effectiveness. In comparative tests made in attics, however, SG 67 at less than one-sixth the weight was about three times as effective, based on the period required to kill termites, as Olancha Clay near the point of discharge of the dust. The ratio of its superiority increased as the distance of the test blocks of wood from the point of discharge was increased, reaching in some cases a ratio of 9 to 1. This reflects not only the greater inherent lethality of SG 67 but also the advantages resulting from its much lower bulk density. The bulk density of Olancha Clay is about 6.5 times as great as that of SG 67. As a result, the latter is more uniformly distributed throughout the attic, reaching the most remote areas in sufficient quantity to kill the termites rapidly. Also it has unusually great ability to adhere to the wood.

In addition to having certain desirable physical characteristics, the silica gels and aerogels have the fluoride coating, which causes them to have considerable toxicity to insects, and this advantage increases with increasing relative humidity, as explained in the companion paper (pages 190 to 207). This added toxicity is not required for the prevention of drywood termites, but may be essential for the control of certain other insect species.

Test blocks of wood were located in all parts of the attics in which field tests were conducted. Some blocks were placed as far as 50 feet from the duster in side gables of the attics, suspended behind rafters and other structures, or wedged up between rafters and ridge poles immediately beneath the roof sheathing. In these cases the dust was sometimes required to make several changes of direction before reaching the blocks. Depending on the location of test blocks, SG 67 gave a 100% kill of drywood termites confined with them in beakers in from 2 to 6 hours when used at 1 pound to 1000 sq. ft. of attic area.

The points most susceptible to entry by drywood termites are cracks under shingles or other openings through the roofing materials and attic vent areas through which swarmers may enter. Field trials showed that wherever there are openings, the dust-laden air will deposit a coating as it escapes to the outside. In dustings of homes, many have appeared to be on fire as the dust billowed out from under shingles and through attic vents, giving the appearance of smoke.

Field trials are being continued and the results thereof will be reported in a later paper. However, pending a final field evaluation of all candidate materials, SG 67 is being recommended for use at the rate of 1 pound per 1,000 sq. ft. of attic area, with the precautions normally taken when applying a dust, namely, the use of a dust respirator.

Although finely divided silica dusts, if they are of a crystalline structure, can cause silicosis when inhaled in sufficient quantity, this is not true of the amorphous silica aerogels, silica gels, and silica precipitates that have been used in the present investigation. Whenever pharmacological studies have been made with the usual test animals, the amorphous silica materials, including those that are impregnated with fluorides, have proved to be harmless. Further tests are being made with the specific materials that currently show the most promise for the dusting of wood structures for the prevention of attack by drywood termites. Even with this assurance, the dusts are irritating to breathe and the operators have worn respirators during all field applications.

Table 2.—Average period required for 100% immobility and 100% mortality of drywood termites placed on blocks of wood dusted in a wind tunnel.

Material	Immobility (Hours)	Mortality (Hours)
Diatomites		
Celite 209	2.33	4.25
Diacron C	6.5	>8.0
#M51 Diatom. Earth	7.0	>8.0
Dicalcite IG-1	3.9	6.7
Dicalcite IG-3	5.4	>8.0
Silica Gels		
Hi-Sil 233	4.25	>8.0
Hi-Sil X-303	2.9	4.9
Santocel C	3.9	6.1
Santocel 54	3.7	6.7
SG 72	4.5	6.3
SG 971	5.3	7.3
SG 976	4.0	6.9
SG 68	2.8	4.8
SG 67	2.7	5.3
SG 77	2.9	>8.0
SG 74	6.2	>8.0
SG 97	2.2	4.2
SG 78	2.2	5.0
Talcs		
#1718 Fibrous Mineral Filler	2.8	8.0
Snythetic Calcium Silicates		
Microcel E	5.8	>8.0
Silene EF	3.5	6.6
Clays (Montmorillonite)		
Olancha Clay	2.3	4.25
Olancha Clay (micron)	4.7	>8.0
Pike's Peak Clay 96-66	6.0	>8.0
Clays (Kaolinites)		
Barden Clay	3.0	5.3
"L" China Clay	8.0	>8.0
Clays (Attapulgites)		
Attaclay	4.9	>8.0
Diluex A	4.8	>8.0
Hi-Dry (calcined)	3.0	6.0
Volcanic Materials		
#181 Feldspar	5.2	>8.0
L.S.D. at P=0.05	2.0	1.8[a]

[a] Does not include materials for which 100% mortality occurred in more than 8 hours.

Acknowledgments.—The authors wish to thank the many firms that contributed materials. This investigation would not have been possible without their cooperation. The following is a list of the contributing firms. The numbers shown here are given in parentheses in table 1 to show which firm contributed each diluent.

(*See list following page*)

No.	Company
1.	Agrashell Inc., Los Angeles, Calif.
2.	American Colloid Co., Chicago, Ill.
3.	American Cyanamid Co., New York, N. Y.
4.	American Tripoli Corp., Senucca, Mo.
5.	Archer Daniels Midland Co., Minneapolis, Minn.
6.	Brown Co., Boston, Mass.
7.	E. S. Browning, Los Angeles, Calif.
8.	California Industrial Minerals Co., Friant, Calif.
9.	California Spray-Chemical Corp., Richmond, Calif.
10.	Carbola Chemical Co., Natural Bridge, N. Y.
11.	Central Soya Co., Decatur, Ind.
12.	Cohutta Talc Co., Dalton, Ga.
13.	Columbia Southern Chemical Co., Pittsburgh, Pa.
14.	Davison Chemical Co., Baltimore, Md.
15.	DEGESCH, Frankfurt, Germany
16.	Dicalite Div., Great Lakes Carbon Corp., Walteria, Calif.
17.	E. I. duPont deNemours & Co., Wilmington, Delaware
18.	Eastern Magnesia Talc Co., Burlington, Vt.
19.	General Reduction Co., Chicago, Ill.
20.	Georgia Talc Co., Chatsworth, Ga.
21.	Glendon Pyrophyllite Co., Greensboro, N. C.
22.	Hamill & Gillespie, Inc., New York, N. Y.
23.	Industrial Mineral & Chemical Co., Berkeley, Calif.
24.	International Talc Co., New York, N. Y.
25.	Johns-Manville Co., Los Angeles, Calif.
26.	Johnson-Marsh Corp., Philadelphia, Pa.
27.	H. E. Millard Lime Co., Annville, Pa.
28.	Mississippi Lime Co,. Ste. Genevieve, Mo.
29.	Monsanto Chemical Co., St. Louis, Mo.
30.	Niagara Chemical Co., Richmond, Calif.
31.	Ohio Lime Co., Woodville, Ohio
32.	Parocel Corp., Menlo Park, N. J.
33.	L. A. Salomon & Bro., New York, N. Y.
34.	Sierra Talc and Clay Co., Pasadena, Calif.
35.	Southeastern Clay Co., Aiken, S. C.
36.	A. E. Staley Mfg. Co., Decatur, Ill.
37.	Stauffer Chemical Co., New York, N. Y.
38.	Tamms Industries Co., Chicago, Illinois
39.	Thompson Weinman & Co., New York, N. Y.
40.	R. T. Vanderbilt Co., New York, N. Y.
41.	Charles A. Wagner, Philadelphia, Pa.
42.	Western Talc Co., Los Angeles, Calif.
43.	Whittaker, Clark & Daniels, New York, N. Y.
44.	Attapulgus Clay Co., Philadelphia, Pa.
45.	Floridin Company, Tallahassee, Fla.
46.	Blue Ridge Talc Co., Inc., Henry, Va.
47.	Waverly Petroleum Products, Philadelphia, Pa.

A Comparison of the Toxicity of Various Insecticides to the Spruce Budworm[1]

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ABSTRACT

While DDT in oil solution is widely and successfully used against many forest insects at the rate of 1 pound in 1 gallon per acre, little is known of the relative effectiveness of other insecticides for this purpose. Laboratory tests are described in which BHC with and without an extender, aldrin, dieldrin, and endrin were compared with DDT in their effectiveness against spruce budworm (*Choristoneura fumiferana* (Clem.)). In some tests larvae on foliage were treated while in others foliage was treated then weathered before larvae were placed in it. Each insecticide was applied in two dosages. In every test DDT was superior.

There is a wealth of information available on the toxicity of various insecticides to agricultural and household insects, but much less is known of their toxicity to forest tree defoliators. This lack of information stems largely from the fact there has been little incentive for testing them, since DDT used at the rate of only 1 pound in 1 gallon of oil per acre is highly effective for the control of many of the defoliators, including the spruce budworm (*Choristoneura fumiferana* (Clem.)). In addition to being effective, DDT has also been considered relatively safe at this dosage for use under forest conditions. Nevertheless, it has been realized all along that other insecticides might exist which are as good or better for control of forest defoliators.

This paper presents the results of a series of tests in which four insecticides—BHC with and without an extender, aldrin, dieldrin, and endrin were studied in the laboratory and compared with DDT in their ability to kill spruce budworm larvae. These insecticides were tested because they were reported to be effective against other insects, easily available, and suitable for application by airplane.

PROCEDURE.—The standard for insecticide comparison was a 12% DDT solution (1 pound DDT per gallon of oil) similar to that commonly used in aerial spraying of forests. Other insecticides were (1) BHC at 4% gamma isomer in fuel oil, and also in a xylene emulsion with Aroclor 5460 (Monsanto Chem. Co.); (2) aldrin at 3.16% in fuel oil; (3) dieldrin at 1.8% in xylene-fuel oil mixture; and (4) endrin at 1.4% emulsion.

A laboratory spray chamber (Eaton & Secrest 1951) was used for applying the insecticides. In this device spray drops are sorted according to size, so that the deposits in any part of the chamber are composed of a narrower range of drop sizes than is possible with a settling tower type spray chamber. In these tests, the spray droplets were of moderate size. Spray atomization, determined by the method described by Davis (1949), was approximately 120 microns mass median diameter. An application rate of 1 gallon per acre was used in all tests. Deposit analyses were made by means of a dye tracer in the spray solution and an optical colorimeter.

A total of five tests were made at a temporary field laboratory of the Northeastern Forest Experiment Station at Ft. Kent, Maine. Uninfested balsam fir foliage and budworm larvae were collected as needed from nearby forest stands. The basic unit for the tests was a 6-inch twig held in a shell vial of water by either a cotton plug or

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FIG. 1.—Unit for testing spruce budworm mortality resulting from insecticides.

a short piece of rubber tubing. In all tests five larvae per unit were used. The period of time over which the tests were run made it necessary to use larvae ranging from the fourth to the sixth instar. In each of the five tests the number of larvae in each of these instars was the same for all treatments.

In three of the tests, the initial effectiveness of the insecticides was determined by applying the spray to foliage that had been infested with larvae 1 day in advance of treatment so that the larvae could become settled and begin feeding. It was the purpose of these tests to simulate as closely as possible typical aerial spraying conditions. Each fir twig was laid in a cardboard meat tray (fig. 1) to which had been applied a border of shellac, topped by tanglefoot to prevent escape of larvae. The trays were then taken to an unheated room and placed on a tilted rack to prevent water from leaking from the vials. The infested twigs were treated by placing them, in their normal growing position, individually, beneath the opening in the spray chamber.

After exposure to the spray, the twigs were returned to the cardboard tray, and any larvae escaping during the spraying were put back with a brush. The trays were stored on laboratory racks until mortality counts were made on the seventh day after spraying. In each test, usprayed units were used as controls to determine mortality from causes other than the treatment.

Two tests were made for comparison of the weathering qualities of deposits. Uninfested units were sprayed, using the same procedure as above, with all units in one test being sprayed on the same day. These were then placed on racks outdoors for exposure to the weather. Unsprayed controls were included as before. Five units were brought into the laboratory and infested with larvae after 3 days of weathering, five after 1 week, and five more after 2 weeks. Once they were infested they also were kept in the laboratory in cardboard trays on tilted racks, and mortality counts were made on the seventh day.

Table 1.—Mean per cent of spruce budworm mortality resulting from various insecticides, corrected for natural mortality.

TEST	MORTALITY PER CENT WHEN INSECTICIDE WAS—					
	DDT (12%)	BHC (4%)	BHC (4%) with Aroclor	Endrin (1.4%)	Dieldrin (1.8%)	Aldrin (3.16%)
Initial effectiveness:[a]						
Test I	55	46	22	0	0	0
Test II	97	81	61	61	0	9
Test III[b]	93	11	—	0	—	—
Effectiveness of weathered deposit:[c]						
Test IV						
3 days	94	63	27	11	0	—
1 week	100	35	33	4	12	0
2 weeks	96	13	10	7	1	—
Test V						
3 days	95	51	63	50	4	0
1 week	95	5	0	0	0	—
2 weeks	50	26	11	13	10	—

[a] Both larvae and foliage sprayed. Number of twig units for each insecticide: test I, 6; test II, 14; test III, 15.
[b] Concentration of insecticide reduced to one-fourth.
[c] Larvae placed on foliage after deposits had weathered for specified lengths of time Five twig units were used for each insecticide in these tests.

Counts were made of the numbers of living and dead larvae in each test. Escaped larvae were not included in the counts. Mortality was calculated according to the method described by Abbott (1925), and results are given in table 1. To test the statistical significance of the treatment differences, the per cent mortalities for individual units (not shown in table) were subjected to an analysis of variance.

RESULTS.—DDT proved to be superior for killing budworm larvae in every test. In tests I and II where DDT was used at 1 pound per gallon, the difference between DDT and BHC was not significant at the 5% level but each was significantly better than the other materials.

In test III where insecticide concentrations were 25% of those in tests I and II, DDT was significantly better than the others. Differences between BHC, endrin, and the unsprayed controls were not significant. In tests IV and V where insecticide deposits were subjected to weathering for periods of 3 days, 1 week, and 2 weeks before larvae were introduced, DDT was significantly better than each of the other insecticides with one exception. In some of the 3-day-old deposits of DDT and BHC, the differences were not always significant.

Differences in the ages of larvae used in tests I and II probably were responsible for observed differences between those two tests. For example, a much higher proportion of sixth-instar larvae was used in test I than in test II. The greater resistance of sixth instar larvae agrees with observations of Godwin (unpublished data) and of Canadian researchers (Hurtig & Rayner 1953a, 1953b, Rayner & Hurtig 1953).

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Studies with P^{32}-Bayer 21/199 Sprayed on Cattle[1]

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ABSTRACT

The metabolism, excretion, and tissue distribution of P^{32}-labeled Bayer 21/199 have been studied following spray application of two Hereford bulls. Only low levels of radioactive compounds were found in the blood, and these behaved like polar degradation products. The compound appears to have been sparingly absorbed, about 2.4 (suspension) and 6.3% (emulsion) of the applied dose being accounted for in the urine of the two animals 2 weeks after treatment. At that time only very low levels of organo-soluble compounds which behaved like 21/199 were present in the tissues, but a considerable residue of unchanged 21/199 was present externally.

The organophosphorus compound Bayer 21/199 has been demonstrated an effective insecticide for the control of encysted cattle grubs (Smith & Richards 1954, Roth & Eddy 1955). Recent research by Brundrett *et al.* (1957) has demonstrated that this compound, when applied as a spray, may also prevent the development and/or encystment of migrating grubs. To investigate the absorption and metabolism of this compound by cattle, P^{32}-labeled Bayer 21/199 was sprayed on two animals at Corvallis, Oregon. Samples of blood and urine were collected and assayed for radioactive compounds, and after 2 weeks the animals were sacrificed and tissue samples taken for analysis.

Materials and Methods.—Radioactive Compound.—The P^{32}-labeled 21/199 (*O*-(3- chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), was synthesized by Farbenfabriken Bayer of Germany. The specific activities of the radioactive compounds were 3.41 and 6.29×10^8 cpm/gm. for the emulsion and suspension treatments, respectively. Both preparations had a radiochemical purity of 98% as determined by paper chromatography. The corresponding phosphate of 21/199 (*O*-(3-chloro-4-methyl-umbelliferone) *O,O*-diethyl phosphate) was also labeled to facilitate the development of methods of solvent partition, extraction, and paper chromatography. This compound had an observable specific activity of 7.67×10^8 cpm/gm.

Spray Application.—Two Hereford bulls were used in these studies. One animal was sprayed with 1 liter of an emulsion and the other with 1 liter of a suspension prepared from a wettable powder formulated by Farbenfabriken Bayer. The animals were treated in a plastic-lined spray chamber with a metal floor, using a 3-gallon compressed-air sprayer. Following treatment the runoff and sprayer and spray-chamber rinses were pooled and assayed radiometrically, and the applied dose was calculated. The treatment data are given below:

	Emulsion-Treated Animal	*Suspension-Treated Animal*
Weight	206 kg.	267 kg.
Formulation	20% emulsifiable concentrate (xylene-Trition X-100)	30% wettable powder
Spray	1.5% P^{32}—21/199	1.5% P^{32}—21/199
Runoff	4.3 gm. (28.7%)	5.1 gm. (34.0%)
Applied dose	10.7 gm. 51.9 mg./kg.	9.9 gm. 37.1 mg./kg.

Sample collection.—The treated animals were held in a metabolism stall similar to that described by Hansard (1951), in a ventilated unheated barn. The temperatures inside the barn were 31 to 71° and 40 to 62° F. for the emulsion and suspension experiments, respectively. Food and water were made available throughout the experimental period. Care was taken to prevent the animals from ingesting the dermally applied insecticide or contaminated bedding. Near-quantitative collection of the urine was maintained by means of an attached urinal (Hansard 1951). Blood samples were taken at intervals. Two weeks after treatment the animals were sacrificed and tissue samples were taken for analysis.

Analyses.—Radioassay was performed with a conventional scaler and a windowless gas-flow proportional counter. All samples were prepared in duplicate, and the mean counting rates corrected for background, decay, and self-absorption, and converted to microgram-equivalents of Bayer 21/199. The methods employed for sample preparation have been previously reported (Robbins *et al.* 1956).

The following chromatographic systems were used in this study:

(1) Reverse-phase (Metcalf & March 1953). Whatman No. 1 paper was coated with a 5% (by volume) solution of the stationary-phase, Dow-Corning Silicone 550, in acetone. The mobile phase was a mixture containing 60 parts of water, 38 parts of absolute ethanol, and 2 parts of chloroform (by volume). This solvent was discarded after two runs.

(2) Carbowax-300 system. Strips of Whatman No. 1 paper were coated with the stationary phase by passing them through a 20% (by volume) acetone solution of

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Carbowax-300. The developing solvent was isopropyl ether containing 0.2% of concentrated ammonium hydroxide (by volume) and 0.001% of resorcinol (by weight). The chamber was lined with Whatman No. 1 paper to insure saturation of the atmosphere with the mobile phase.

(3) Pyridine-ammonium hydroxide system (Robbins *et al.* 1957). This system was employed in the separation of urine metabolites.

Glass-stoppered graduated cylinders (500 ml.) containing 50 ml. of the developing solvent were employed as chambers. A 20- to 30-cm. development by the ascending method was employed for all three systems.

The Rf values for Bayer 21/199 and related compounds determined by these systems are reported in table 1. These were determined colorimetrically and/or radiometrically. Bayer 21/199 and its corresponding phosphate were detected with ammoniacal silver nitrate and UV light (Price & Dietrich 1956). Diethyl phosphorothioic and diethyl phosphoric acids gave color development with acid permanganate and by the method of Hanes & Isherwood (1949), respectively. Radiometric analyses were made by cutting the developed chromatograms into 1-cm. strips and assaying in a windowless proportional counter.

Blood samples were assayed for organosoluble radioactive compounds by thoroughly shaking with equal volumes of isopropyl ether in a stoppered, graduated centrifuge tube. After centrifuging, duplicate samples of the isopropyl ether layer were plated and assayed radiometrically. When P^{32}-labeled 21/199 and the corresponding phosphate were added to the control blood and treated in the same manner, 97.4% of the 21/199 and 85.7% of the phosphate (means of three determinations) were found to partition into the isopropyl ether.

Total radioactive compounds in the tissue were determined by homogenizing 100 gram samples, previously passed through a food chopper, in a Waring Blendor. Aliquots of the homogenate were plated, air-dried, and assayed radiometrically.

Organosoluble compounds present in liver, kidney, and fat were determined by blending 50-gram samples, previously passed through a food chopper, with 100 ml. of isopropyl ether. The extracts were centrifuged, dried over anhydrous sodium sulfate, filtered, and made up to volume, and aliquots were plated for radioassay. When tissues to which 21/199 or its phosphate had been added were treated in this manner, 94.0% of the 21/199 and 89.0% of the phosphate were recovered (means of two determinations). These extracts were "cleaned up" by evaporating the isopropyl ether with a gentle stream of air; the extractives were taken up in hexane and extracted with three equal volumes of acetonitrile saturated with hexane. The first extraction removed 96.4% of the 21/199 and 98.9% of its phosphate (means of three determinations), and two additional extractions with acetonitrile resulted in quantitative extraction of both compounds.

Acetone precipitation of the phospholipids from the liver and kidney isopropyl ether extractives was accomplished by the method of Borgstrom (1952). When P^{32}-21/199 or its phosphate was added to these extractives and the phospholipids were precipitated with acetone, 99.4% of 21/199 and 97.4% of the phosphate were recovered in the acetone supernatant.

Table 1.—R_f values for Bayer 21/199 and related compounds at 23–25° C., as determined by different chromatographic systems.

Compound	Silicone Reverse-Phase	Carbowax-300	Pyridine-Ammonia Hydroxide
Bayer 21/199	0.04	0.69	0.84
Phosphate of Bayer 21/199	.51	.23	.84
Diethylphosphorothioic acid	.89	.00	.66
Diethylphosphoric acid	.90	.00	.45

The P^{32}-labeled compounds in the liver and kidney were fractionated according to the method of Schneider (1945).

Cholinesterase activity was measured by the colorimetric whole-blood method of Fleisher & Pope (1954) as modified for the determination of bovine RBC ChE activity (Robbins *et al.* 1958).

RESULTS.—*Blood.*—The blood data are summarized in table 2. Only low levels of total radioactive compounds were found in the blood of either animal. The emulsion-treated animal attained a maximum approximately four times that found for the animal treated with the suspension. When these blood samples were extracted with equal volumes of isopropyl ether, none of the radioactivity behaved like the parent compound or its corresponding phosphate. The method was sensitive to 0.035 μg./ml. at the beginning and 0.070 μg./ml. at the end of the experimental periods.

Measurement of the RBC ChE activity demonstrated inhibition of about 35% in both animals after 2 weeks (table 2).

Urine.—Apparently only a small percentage of the radioactivity representing the dermally applied 21/199 was absorbed and excreted via the urine (table 3). Both the levels of radioactive compounds and the percentages of the dose excreted were about 2.6 times greater in the emulsion-treated animal. Paper chromatographic analyses were run on the sample containing the highest concentration of radioactive compounds, 5- and 6-day composite of the emulsion-treated animal, by the pyridine-ammonia hydroxide and reverse-phase systems. Only traces of the radioactivity behaved like unchanged

Table 2.—Radioactive compounds in blood and RBC Ch E activity of Hereford bulls following spray application of P^{32}-Bayer 21/199.

Days	Emulsion-Treated Animal		Suspension-Treated Animal	
	μg.-equiv./ml.	Per Cent Control of RBC Ch E activity	μg.-equiv./ml.[a]	Per Cent Control of RBC Ch E activity
1	0.05[a]	98.0	0.04	79.3
2	.06[a]	81.5	.06	70.7
3	.08[a]	73.5	.05	71.7
4	.07[a]	74.2	.05	69.6
6	.27	58.3	.06	65.2
8	.20	60.3	.06	66.3
10	.22	59.6	.05	66.3
12	.25	57.6	.05	67.4
14	.23	65.5	.05	64.1

[a] Standard error greater than ±5%.

Table 3.—Radioactive compounds in urine of bulls following spray application of P^{32}-Bayer 21/199.

Days	Emulsion-Treated Animal		Suspension-Treated Animal	
	μg.-equiv./ml.	Cumulative Per Cent of Applied Dose	μg.-equiv./ml.	Cumulative Per Cent of Applied Dose
1	8.3	0.14	4.3	0.15
2	7.8	.41	7.9	.35
3	5.8	.66	8.3	.61
4	6.5	.91	6.8	.75
5	20.4	1.73	6.6	.94
6	22.8	2.74	8.3	1.30
7	11.0	3.31	8.8	1.54
8	8.4	3.70	6.6	1.71
9	8.6	4.08	5.3	1.86
10	7.4	4.44	4.0	1.99
11	11.9	4.96	4.0	2.09
12	13.0	5.52	2.9	2.20
13	9.9	5.93	2.9	2.33
14	10.0	6.27	2.8	2.44

21/199. About 42% of the radioactive compounds ran with the Rf of diethyl phosphorothioic acid and 18% with the Rf of diethyl phosphoric acid (means of three chromatograms) by the pyridine-ammonium hydroxide system. The rest of the radioactivity, representing more polar degradation products, remained near the point of application.

The feces were checked at intervals and found to contain low levels of radioactivity.

Tissues.—Of the internal tissues analyzed, the liver and kidney contained the highest concentrations of radioactive compounds in both animals (table 4). The maximum residues of radioactive compounds were found externally.

A 50-gram sample of hide from the suspension-treated animal was rerinsed several times with acetone and then extracted with isopropyl ether. The rinse contained 245.6 and the extract 1.2 μg.-equiv./gm. Paper chromatographic analysis of the acetone rinse demonstrated that 98% of the radioactive compound(s) ran with the Rf of 21/199.

Extraction of the homogenates of the tissues with an equal volume of isopropyl ether demonstrated that only the liver and kidney contained significant amounts of organosoluble radioactive compounds. These two tissues and samples of omental, perirenal, and subcutaneous fat

Table 4.—Total radioactive compounds in tissues 2 weeks after spraying with P^{32}-Bayer 21/199, as determined from homogenates.

Tissue	Microgram-Equivalents per Gram	
	Emulsion-Treated Animal	Suspension-Treated Animal
Liver	10.84	4.12
Kidney	23.39	3.26
Muscle	.31	.08
Brain	.41	.19
Spleen	—	.16
Hide	—	607.6[a]

[a] Determined from wet-ashed samples, mean of five 1-gram samples, range 26.7-1,525.4 μg./g.

Table 5.—Isopropyl ether-soluble radioactive compounds in tissue and extraction of these compounds from a hexane solution with three equal volumes of acetonitrile.

Tissue	Emulsion-Treated Animal		Suspension-Treated Animal	
	Isopropyl Ether Extract μg.-equiv./ml.	Per Cent Extracted with Acetonitrile	Isopropyl Ether Extract μg.-equiv./ml.	Per Cent Extracted with Acetonitrile
Liver	0.13	12.0	0.03	12.1
Kidney	.43	4.5	.09	8.1
Fat:				
Subcutaneous	0[a]	—	.14	78.6
Perirenal	0[a]	—	0[a]	—
Omental	0[a]	—	0[a]	—

[a] Less than 0.08 μg.-equiv./gm.

were extracted with isopropyl ether as previously described, and the results are given in table 5.

The highest concentration of isopropyl ether-soluble radioactive compounds, 0.4 μg.-equiv./gm., was found in the kidney of the emulsion-treated animal. The liver of this animal and the kidney and subcutaneous fat of the other animal all contained about 0.1 μg.-equiv./gm. When these compounds were taken up in hexane, and the hexane solution was extracted with three equal volumes of acetonitrile, only 4.5% of the organosoluble radioactive compounds in the liver and 12% of those in the kidney partitioned like 21/199. More than 78% of the radioactivity in the subcutaneous fat sample of the suspension-treated animal was extracted with acetonitrile, giving a concentration of 0.11 μg.-equiv./gm. The sensitivity for the fat-analysis technique, which was limited by the self-absorption of the large quantity of extractives, was about 0.08 μg.-equiv./gm.

To investigate the nature of the radioactive organosoluble compounds in the liver and kidney, the extractives in the hexane and acetonitrile fractions from the suspension-treated animal were analyzed by acetone precipitation. About 23 and 10% of the radioactivity in the acetonitrile fraction and 96 and 85% in the hexane fraction of the liver and kidney, respectively, behaved like phospholipids (table 6).

When the P^{32}-labeled compounds in the liver and kidney of this animal were fractionated according to the method of Schneider (1945), more than one-half the P^{32} in these tissues was in the phosphoprotein fraction (table 7). Appreciable quantities of radioactive phosphorus were also present in the acid-soluble phosphates and phospholipids, with the lowest levels in the nucleic acid fraction of both tissues.

Discussion.—The limited excretion of radioactive

Table 6.—Acetone precipitation of the phospholipids in the isopropyl ether extracts of the liver and kidney of animal treated with a suspension of Bayer 21/199.

Tissue	μg.-Equiv./Ml.	Per Cent in Phospholipid Precipitate	Per Cent in Acetone Supernatant
	Hexane Fraction		
Liver	0.026	96.0	4.0[a]
Kidney	.083	85.0	15.0[a]
	Acetonitrile Fraction		
Liver	0.004	23.3[a]	76.7
Kidney	.007	10.0[a]	90.0

[a] Standard error greater than ±5%.

Table 7.—Fractionation of the radioactive phosphorus in the liver and kidney of suspension-treated animal.

FRACTION	PER CENT OF TOTAL P32	
	Liver	Kidney
Acid-soluble phosphate	20.2	23.9
Phospholipid	11.8	18.7
Nucleic acid	1.5	5.2
Phosphoprotein	66.5	52.2

compounds and the high external residues 2 weeks after treatment indicate that Bayer 21/199 is only sparingly absorbed following spray application when the animals are prevented from ingesting the dermally applied insecticide or contaminated bedding. Both the pattern of excretion and the dissipation of external residues for this compound differ markedly from those found for malathion (March *et al.* 1956).

Higher blood and tissue levels and excretion rate were found in the emulsion-treated animal, which, although it received approximately the same total dose of 21/199, received 1.4 times as much on a mg./kg. basis as the animal sprayed with the suspension. It is not known whether these differences were due to the formulation or to individual differences in experimental animals.

One metabolic pathway following dermal application, as determined from urine metabolites, appears to be by enzymic hydrolysis to form the more polar degradation products, diethylphosphorothioic and diethylphosphoric acids. The latter could be formed by enzymic oxidation before or after hydrolysis. The enzymic oxidation of 21/199 to its corresponding phosphate in the white mouse has been demonstrated both *in vivo* and *in vitro* with liver preparations (Robbins *et al.* 1958). Some of the 21/199 is completely metabolized to inorganic phosphorus, which enters the metabolic pool and is utilized by the bovine in the syntheses of normal phosphorus-containing compounds. The incorporation of radioactive phosphorus in the phospholipids following administration of a P32-labeled insecticide re-emphasizes that care must be taken in evaluating residue data where only extraction plus radiometric assay is employed in analysis.

The highest concentration of organosoluble radioactive compound that partitioned like Bayer 21/199, about 0.1 μg.-equiv./gm., was found in the subcutaneous fat of the suspension-sprayed animal. Partition and acetone precipitation of the organosoluble radioactive compounds in the liver and kidney demonstrated that only trace amounts, less than 0.02 μg.-equiv./gm., behaved like unchanged toxicant. Because of the very low level of radioactivity and/or the large quantity of extractives present even after "cleanup," chromatographic analyses could not be made on these samples.

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Interactions of Topically Applied Piperonyl Butoxide and Malathion in Producing Lethal Action in House Flies[1]

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ABSTRACT

The nature of the antagonistic effects of piperonyl butoxide to the toxic action of malathion on house flies, *Musca domestica* L., was investigated. Malathion was topically applied to various sites on the house fly body with varying degrees of toxicity. The same phenomena occurred for the relative toxicity of malathion in combination with piperonyl butoxide.

The antagonistic effects of piperonyl butoxide were demonstrated even when it was applied on a separate area of the integument, separated from malathion treatment site, in nearly simultaneous topical applications to individual flies.

The antagonistic effects of piperonyl butoxide decreased slowly over a 30-hour period, when it was applied first, followed at varying time intervals by the toxicant. In the reverse order of treatment such antagonistic effects decreased rapidly over a range of 30 minutes between the applications of malathion and piperonyl butoxide. The application of piperonyl butoxide 1 to 2 hours before malathion markedly increases the magnitude of the antagonism observed for simultaneous applications.

The antagonistic effects of piperonyl butoxide to the lethal action of malathion in a DDT-susceptible and a resistant strain of house flies were reported by Rai *et al.* (1956). In these studies, malathion plus piperonyl butoxide mixtures (1:10 ratio by weight) were topically applied on the mesosternum of individual flies. The application of toxicants and toxicant-synergist mixtures to various designated areas of insect integument produces different levels of toxicological symptoms, either leading to death or enhanced degrees of knockdown (Bodenstein 1946, Wilson 1949, Fisher 1952, LeRoux & Morrison 1954). Lindquist *et al.* (1947) presented the results of pretreating house flies with synergists before applying pyrethrum sprays. Longevity of the synergistic effects of piperonyl butoxide to pyrethrum for house flies in residual panel studies was reported by Chamberlain (1950). Since the antagonistic effects of piperonyl butoxide to the action of malathion was an uncommon occurrence, further investigations of the interactions of topically applied piperonyl butoxide and malathion at varying sites and in varying combinations were conducted.

EXPERIMENT I: EFFECT OF SITE OF APPLICATION ON THE TOXICITY OF MALATHION IN KUN STRAIN OF HOUSE FLY.—*Materials and Methods.*—Four-day-old adult male house flies of a strain designated KUN were employed in this investigation. This strain has been reared in the laboratory with no previous history of insecticidal exposure, and is known to be a DDT-susceptible strain. These flies were reared in accordance with standard CSMA (Chemical Specialties Manufacturers Association) procedures modified only with reference to the adult diet. Adult flies were provided with water and a mixture of non-fat powdered milk (2 parts by volume) and cane sugar (1 part by volume) in separate paper dishes. The rearing room was maintained at a temperature of 80 ± 2°F.

The flies were immobilized under a minimum CO_2 flow to facilitate handling during sexing and treating. The treatment of the flies was conducted in a separate room maintained at a temperature of 80 ± 3° F., where they were held for 24 hours for mortality counts.

A solution containing 0.25 μg. of 99.6% pure malathion per μl. of glass-distilled acetone, was prepared just prior to making each treatment. This solution at 1.07 ± 0.05 μl. was applied by a microdevice (Roan & Maeda 1956). One group of 25 flies, replicated six times, was treated in each of the four areas: proboscis and head; thoracic sternum; notum; and middle of abdominal sternum. The possibility of slight amount of spreading of the drops over neighboring areas was not entirely eliminated. Following treatment, the flies were placed in wide mouth, glass pint jars, provided with liquid milk, and held for mortality observations. For each of the four groups controls consisted of two replicates of 25 flies each, which were treated with glass-distilled acetone in the same manner as the other groups. Control mortalities were corrected for by Abbott's formula (Abbott 1925). Periodical counts were taken for the dead and live house flies in various groups but the final counts at 24 hours after treatment have been used in this paper.

Results and Discussion.—The corrected and average per cent mortality of various replicates for the different treatments are presented in table 1. The maximum toxic effect of malathion occurred when applied either on the proboscis and head or on the thoracic sternum (the difference between 47.33 and 46.00% was not significant). But there existed a significant difference in the level of toxicity when malathion was applied either to the notum or to the middle of the abdominal sternum. The order of toxicity of malathion to various areas is proboscis and head = thoracic sternum > notum = abdominal sternum.

These data suggest that the closer the malathion is applied to the bulk of central nervous tissue of house flies (brain and thoracic ganglia) the higher is the mortality.

EXPERIMENT II. EFFECTS OF SITE OF APPLICATION ON THE TOXICITY OF MALATHION WHEN APPLIED IN COMBINATION WITH PIPERONYL BUTOXIDE TO KUN STRAIN OF HOUSE FLIES.—*Materials and Methods.*—The same strain was used and was reared and sexed identically to Experiment I. A fresh solution of malathion plus piperonyl butoxide (0.375 μg. of malathion + 3.75 μg. of piperonyl butoxide per μl. of solution was prepared in glass-distilled acetone and applied topically to individual flies at the same areas and under identical conditions as in Experiment I.

Results and Discussion.—The per cent mortalities obtained for the various group replicates are presented in

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Table 1.—Per cent mortality of house flies by applying measured quantities of malathion[a] to different areas of integument.

Loci of Application	Per Cent Mortality in Various Replicates[b]						Average Per Cent Mortality
	1	2	3	4	5	6	
Proboscis and head	52	56	48	60	24	44	47.3
Thoracic sternum	64	36	40	56	52	28	46.0*
Notum	24	36	36	52	20	20	31.3
Abdomen sternum[c]	16	16	24	28	28	28	23.3

L.S.D. = 14.33 at the 5% level.

[a] 0.27 μg. in 1.07 μl acetone solution was applied to each test house fly adult male.

[b] Figures given are corrected for any mortality in control group by Abbott's formula.

[c] The middle of the abdominal sternum.

* Significant.

Table 2.—Per cent mortality of house flies by applying measured quantities of malathion plus piperonyl butoxide (1:10 ratio) to different areas of integument.[a]

Application Sites	Per Cent Mortality in Various Replicates						Average Per Cent Mortality
	1	2	3	4	5	6	
Proboscis and head	44	52	56	68	36	40	49.3[b]
Thoracic sternum	60	24	52	48	64	60	51.3
Notum	32	52	20	40	40	40	37.3[b]
Abdomen sternum	32	28	40	28	32	40	33.3

[a] 1.07 μl. of acetone containing 0.375 μg. of malathion plus 3.75 μg. of piperonyl butoxide was applied at varying sites to each test house fly adult male. This excess was in contrast to 0.27 μg. (table 1) to compensate for antagonism (Rai *et al.* 1956).

[b] Not significant.

table 2. Statistical analysis indicated no significant differences. Apparently, the level of toxicity of malathion in combination with piperonyl butoxide, table 2, obtained for the thoracic sternum, and proboscis and head, are about the same, 51.3 and 49.3%, respectively. The same thing was true, more or less, for malathion alone (table 1) where the difference was also nonsignificant. As far as the relative order of toxicity from applications to the notum and abdominal sternum is concerned, the application of malathion plus piperonyl butoxide to the abdominal sternum resulted in a lower per cent mortality than the application on the notum. This relationship, viz., a higher per cent mortality for flies treated on the notum, is also true in cases where malathion alone is used (table 1). The order of toxicity of malathion in combination with piperonyl butoxide applied to various areas is thoracic sternum = proboscis and head > notum = abdominal sternum.

EXPERIMENT III. EFFECT OF PIPERONYL BUTOXIDE ON THE TOXICITY OF MALATHION WHEN BOTH APPLIED SIMULTANEOUSLY AT DIFFERENT SITES.—This experiment was conducted to determine if the antagonistic effect of piperonyl butoxide to the lethal action of malathion is the result of a decreased rate of penetration of malathion by the presence of piperonyl butoxide in a solution.

Materials and Methods.—Four-day-old adult males of KUN strain were used. Fresh solutions of malathion and piperonyl butoxide were made separately in glass-distilled acetone. Each solution was applied topically to designated areas of individual house flies, using two micro-applicators manipulated side by side. The rates of delivery from the ¼ cc. B. D. Yale tuberculin syringes mounted on the micro-applicators were 1.07 μl. for malathion and 1.03 μl. for piperonyl butoxide solution, the difference being a result of syringe calibration. The concentration of piperonyl butoxide solution was 10 times stronger (weight/volume) than the malathion. Because of differences in delivery rates of the micro-applicators, slightly less than 10 times more piperonyl butoxide than malathion was applied to each fly.

Four different sites of treatments were selected: (1) malathion on mesosternum and piperonyl butoxide solution on middle of abdominal sternum; (2) malathion on middle of abdominal sternum and piperonyl butoxide on the mesosternum; (3) malathion applied on mesosternum; and (4) malathion applied to middle of abdominal sternum. When used in combination, the malathion solution was applied before the piperonyl butoxide solution. Precautions were taken to prevent the overlap of the solutions on the insect integument.

Two replicates of 25 flies each were employed under each treatment. Two control groups of 25 flies each were treated with acetone on the mesosternum and abdominal sternum. There was no mortality in the control groups. Mortality counts were taken 24 hours after the treatment.

Results and Discussion.—There was a 54% mortality in the group where malathion was applied on the mesosternum and piperonyl butoxide on abdominal sternum and only 22% mortality in the group where the application was reversed. This is in contrast to the 70 and 38% mortalities produced in groups in which only malathion was applied to the mesosternum and abdominal sternum, respectively (table 3).

These data strongly suggest that piperonyl butoxide does not slow down the rate of penetration of malathion. It is also interesting to note that the simple arithmetical ratios of per cent mortality observed as the result of malathion alone applied to the mesosternum as compared with this plus piperonyl butoxide on the abdominal tergite, compared with (70/54 = 1.3) the reversed order of treatment (38/22 = 1.7) are almost equal to that reported by Rai *et al.* (1956). These data suggest that the level of antagonism is not dependent on applications of these two materials to the same areas.

Table 3.—Effects of piperonyl butoxide[a] on the toxicity of malathion[b] on the house fly both applied simultaneously but at varying sites.

Loci of Application	Average Per Cent Mortality
Malathion on mesosternum and piperonyl butoxide on abdominal sternum	54
Malathion on abdominal sternum and piperonyl butoxide on mesosternum	22
Malathion on mesosternum	70
Malathion on abdominal sternum	38

[a] 2.58 μg. of piperonyl butoxide solution delivered in 1.03 μl. glass distilled acetone was applied to each fly.

[b] 0.27 μg. of malathion solution delivered in 1.07 μl. glass distilled acetone was applied to each fly.

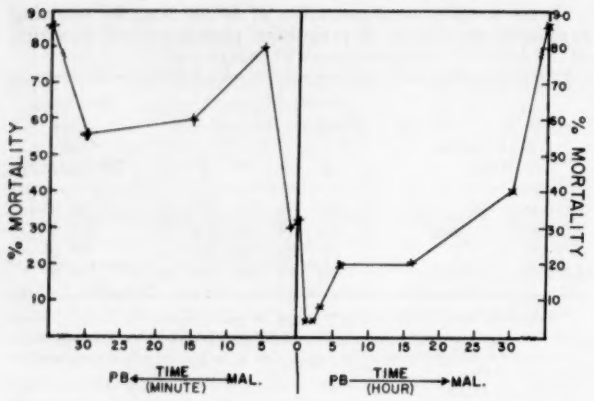

FIG. 1.—The 24-hour mortalities resulting from the application of one compound followed at the indicated intervals by the second compound. Points at either end indicate mortalities from the application of malathion alone. Zero time indicates the per cent mortality resulted from malathion plus piperonyl butoxide mixture treatment in single application.

PB = Piperonyl butoxide, 4.28 μg. of technical material in 1.07 μl. acetone solution applied to each test house fly adult male.

MAL = Malathion, 0.428 μg. of 99.6% pure material in 1.07 μl. acetone solution applied to each test house fly adult male.

EXPERIMENT IV. DURATION OF ANTAGONISTIC EFFECTS OF PIPERONYL BUTOXIDE TO THE LETHAL EFFECTS OF MALATHION.—*Materials and Methods.*—Four-day-old male adult flies of the Campus strain (a DDT-resistant strain) were used. These flies were reared similarly to the KUN strain used in the previous experiments. This Campus strain was approximately twice as tolerant to malathion as the KUN strain, although there was no record of exposure to malathion either in the field or in the laboratory.

Two types of treatments were involved. To one group, piperonyl butoxide solution (4.28 μg./fly) was applied followed by an application of malathion (0.428 μg./fly) at hourly intervals. Different lots of flies were used for each subsequent treatment. To the other group of flies, malathion was applied first followed by an application of piperonyl butoxide at intervals of minutes, at the same concentrations. Both the malathion and piperonyl butoxide were applied to the mesosternum. Control flies received two treatments with acetone at the varying intervals.

All treatments were conducted at a temperature of 80±3° F. Final mortality counts were taken 24 hours after the last application. Two replicates of 25 flies each were employed for each treatment.

RESULTS AND DISCUSSION.—The per cent mortalities resulting from various treatments of house flies with malathion and piperonyl butoxide at various intervening periods are plotted in figure 1. The per cent mortality resulting from a mixture of malathion and piperonyl butoxide (1:10 ratio by weight) was 32% and is plotted on the zero coordinate line. Points at either end of the coordinate lines indicate mortalities from the application of malathion alone.

When piperonyl butoxide was applied to the flies first (right half of figure), its antagonistic effects were existent up to 30 hours or more. The level of antagonistic effect (lower mortality) was higher when the intervals between piperonyl butoxide and malathion treatments were smaller. There is a suggestion that the prior application of piperonyl butoxide, *i.e.*, 1 or 2 hours, produces a greater antagonistic effect than the simultaneous application of piperonyl butoxide and malathion.

In the reversed order of treatments (left half of figure), where malathion treatment was followed by the piperonyl butoxide treatment, the magnitude of the antagonistic effect of piperonyl butoxide rapidly decreases in minutes, rather than hours as indicated for the application of piperonyl butoxide before malathion. The mortalities observed when the malathion application preceded the piperonyl butoxide by 30 minutes are nearly comparable with those observed when the piperonyl butoxide preceded the malathion by 30 hours.

The antagonistic effect of piperonyl butoxide appears to be relatively persistent, gradually decreasing over a period of 30 hours. The intoxication process(es) of malathion, on the other hand, are rapidly initiated and their susceptibility to the antagonistic effects of piperonyl butoxide decreases relatively rapidly.

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Control of Caterpillars on Cabbage in the Ottawa Valley of Ontario and Quebec, 1956–1957[1]

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ABSTRACT

In 1957, 92 to 85% control of the imported cabbageworm, *Pieris rapae* (L.), the diamondback moth, *Plutella maculipennis* (Curt.), and a DDT-resistant strain of the cabbage looper *Trichoplusia ni* (Hbn.), on late cabbage was obtained with four applications of the following insecticides: 1% endrin dust at 0.25 lb. of toxicant per acre, 2½% Guthion dust at 0.75 lb., and Phosdrin emulsible concentrate at 0.75 lb. The cabbage looper was not controlled with four applications of the following: 1% parathion dust at 0.33 lb. of toxicant per acre, 3% DDT dust at 1.0 lb., 4% malathion dust at 1.0 lb., 5% toxaphene dust at 1.5 lb., and 1% rotenone dust at 0.33 lb. In 1956, the eight insecticides gave 97 to 87% control of the imported cabbageworm and the diamondback moth, populations of the cabbage looper being almost negligible. Comparison of present and past effectiveness of DDT did not suggest development of strains of the imported cabbageworm resistant to this material.

In the Ottawa Valley of Ontario and Quebec, cabbage is transplanted from the seedbed to the field in two periods. The early crop, which matures in July, is planted in late May; the late crop, which matures in October, is planted in late June. The early crop frequently escapes serious feeding injury by caterpillars and in most years does not require an insecticide if harvested by the middle of July. If it is harvested during late July a single application of rotenone is required to control larvae of the imported cabbageworm, *Pieris rapae* (L.), and the diamondback moth, *Plutella maculipennis* (Curt.), which increase rapidly in numbers at this time. Populations of the cabbage looper, *Trichoplusia ni* (Hbn.), are negligible on the early crop.

Caterpillars of all three species attack the late crop. The imported cabbageworm is the most important of the three species and causes considerable economic damage each year. The diamondback moth, although usually more numerous than the imported cabbageworm, is less important because it consumes less foliage per larva. The cabbage looper is not sufficiently abundant in most years to cause commercial damage but has been an important pest recently.

Experiments in the Ottawa Valley from 1947 to 1954 excluding 1951 and 1952 showed that almost complete control of caterpillars on late cabbage could be obtained with four properly timed applications of DDT (Matthewman *et al.* 1952, Harcourt & Cass 1955). However, in 1955 the insecticide failed to give satisfactory control because of an outbreak of a strain of the cabbage looper resistant to it (Harcourt 1956). The following year evidence of resistance to DDT by strains of the cabbage looper and the imported cabbageworm was observed in southwestern Ontario (Miller 1956).

Endrin gave satisfactory control of the cabbage looper in experiments at Ottawa from 1953 to 1955 and has given effective control of DDT-resistant strains of the imported cabbageworm in Wisconsin (Chapman 1955) and Connecticut (Wheatley 1955). However, this insecticide has a long residual life and should not be applied to cabbage after the heads begin to form in case the residues exceed legal tolerances. Accordingly, studies were carried out at Ottawa in 1956 and 1957 to determine the value of several less stable insecticides for control of caterpillars on late cabbage.

METHODS AND MATERIALS.—The experimental plots were in growers' fields within 10 miles of Ottawa, at Merivale, Ontario, in 1956, and at Aylmer, Quebec, in 1957. The plots, which averaged one-fiftieth of an acre in size, were arranged in randomized blocks with four replications. The experimental areas measured 3/5 of an acre in 1956 and 4/5 of an acre in 1957. The variety of cabbage in both years was Penn State Ballhead.

Table 1 lists the insecticides and the concentrations at which they were tested. The dusts were applied with rotary hand dusters at average rates of 25 to 40 pounds per acre of the diluted dust. The emulsible concentrate was applied with a knapsack sprayer at 100 gallons per acre of the diluted spray. Four applications were made at 15-day intervals beginning on July 20, 1956, and July 23, 1957. To reduce the possibility of drift the insecticides were applied in the early morning when wind velocities were low, averaging 1.4 m.p.h. in 1956 and 1.2 m.p.h. in 1957. Rainfall in the 48-hour period after each application was negligible.

Population Records.—To determine the relative abundance of the species attacking the plants, population counts were made in the untreated plots on four occasions each year (fig. 1). A leaf-by-leaf examination of 25 plants was carried out on each date; six plants were chosen at random from each of the four untreated plots plus one plant at random from one of them. Although all the immature stages of the species were recorded each year, only the data pertaining to larval populations are presented because they are typical of the populations as a whole. Figure 1 shows that the imported cabbageworm was the most abundant species in the untreated plots each year, being present in peak numbers during early August in 1956 and late August in 1957. The diamondback moth formed about a fifth of the total numbers each year; it was most abundant during late August in 1956 and early August in 1957. The cabbage looper, almost nonexistent in 1956, was present in significant numbers in 1957, being most abundant during late August and early September.

As the feeding ratio of equal populations of the diamondback moth, the imported cabbageworm, and the cabbage looper is 1:7.5:11.6 (Harcourt *et al.*, 1955), the foliage consumptions (based on the product of the num-

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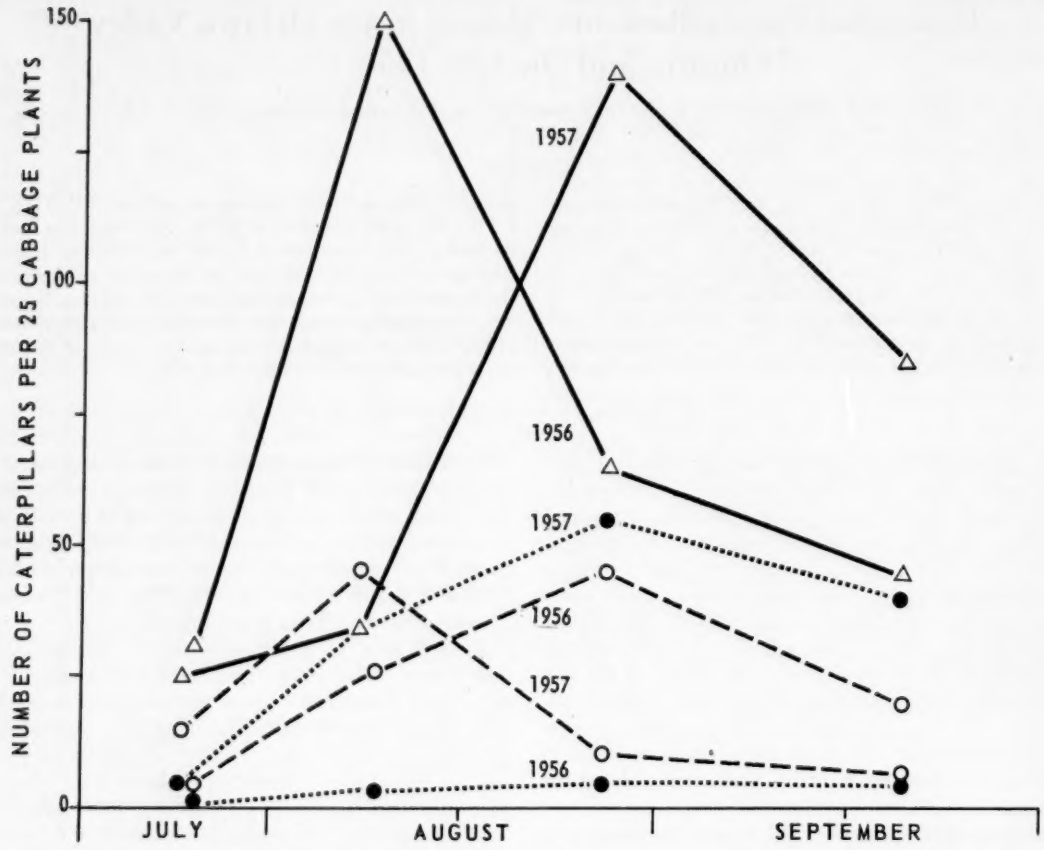

FIG. 1.—Relative abundance of caterpillars on late cabbage, Merivale, Ontario, 1956, and Aylmer, Quebec, 1957. △, imported cabbageworm; ○, diamondback moth; ●, cabbage looper.

ber of larvae recorded and the feeding ratio) attributable to the three species in 1956 and 1957 were in the ratios of 1:23:1 and 1:29:20, respectively. Consequently, the insecticides were evaluated essentially against the imported cabbageworm in 1956 and against the imported cabbageworm in association with the cabbage looper in 1957.

Criterion of Effectiveness.—Effectiveness of the materials was based on the degree of caterpillar feeding injury to the plants at the beginning of harvest, September 26, 1956, and October 2, 1957. The injury records were taken independently by two observers and the results averaged. Excepting the two outside rows and a 3-foot strip at the end of each row which were considered as buffers, the cabbage was graded according to damage to the heads and leaves as follows: *Grade 1*, up to four leaves skeletonized; *Grade 2*, five or more leaves skeletonized. The latter category denoted sufficient injury to reduce or destroy the market value of the crop. A skeletonized leaf was regarded as one in which more than one-sixth of the leaf surface had been removed by feeding.

RESULTS AND DISCUSSION.—Table 1 shows that in 1956 all of the insecticides except rotenone gave excellent control of the caterpillars. However, in 1957, only endrin gave excellent control, Guthion and Phosdrin gave good control, parathion and DDT gave only fair protection, and the remaining materials were unsatisfactory.

A comparison of the present and past effectiveness of DDT in the Ottawa Valley did not suggest development of strains of the imported cabbageworm resistant to this insecticide. The control of the caterpillar species complex afforded by four applications of 3% DDT dust at 1 pound of toxicant per acre in 1956 compared favorably with that obtained from 1947 to 1950, and with that in 1953–1954 when the degrees of infestation of *P. rapae*, schedules of application, and grades of feeding injury to the foliage were essentially the same. The percentages of control obtained from 1947 to 1954 ranged from 96 to 100, averaging 98. However, the performance of DDT in 1957 when the cabbage looper was abundant was only mediocre. It is thus apparent that cabbage growers in the Ottawa Valley using a DDT schedule will need to apply the material at a higher rate, perhaps at 2 pounds per acre, to guard against damage by the cabbage looper. (In a separate experiment at Aylmer, Que., in 1957, four applications of 5% DDT dust at 2 pounds of toxicant per acre gave 85% control.)

Endrin has given good to excellent control of all three species of caterpillars since 1953 at Ottawa, the percentages of control in seven experiments having averaged 94. The present data confirm its value for use before the cabbage heads begin to form.

Guthion and Phosdrin gave excellent control of the imported cabbageworm and good control of the cabbage

Table 1.—Control of caterpillars on late cabbage with four applications of various insecticides, Merivale, Ontario, 1956, and Aylmer, Quebec, 1957.

Insecticide	Formulation	Toxicant per Acre, Lb.	Cabbage Plants: Number Examined 1956	Cabbage Plants: Number Examined 1957	Cabbage Plants: Percentage Grade 2[a] 1956	Cabbage Plants: Percentage Grade 2[a] 1957	Per Cent Control 1956	Per Cent Control 1957
Endrin[b]	1% dust	0.25	421	319	1.9	7.8	96.9	92.0
Guthion[c]	2½% dust	0.75	500	311	3.4	13.5	94.4	86.2
Phosdrin[b]	emul. conc.	0.75	497	303	4.6	14.5	92.5	85.1
Parathion[d]	1% dust	0.33	513	304	2.3	22.4	96.2	77.0
DDT[c]	3% dust	1.00	431	307	2.6	25.7	95.7	73.7
Malathion[e]	4% dust	1.00	421	304	4.0	31.3	93.5	67.9
Toxaphene[f]	5% dust	1.50	497	327	3.4	53.8	94.4	44.9
Rotenone[d]	1% dust	0.33	404	308	9.5	59.4	87.2	39.1
Untreated	—	—	406	210	61.1	97.6	—	—
L.S.D. at 5% level					1.5	1.7		
at 1% level					2.7	3.2		

[a] Five or more leaves skeletonized, September 26, 1956, and October 2, 1957.
[b] Shell Oil Company of Canada, Toronto 1, Ont. The Phosdrin contained 12.3 lb. of toxicant per Imperial gallon.
[c] Green Cross Products Division, Sherwin-Williams Co. of Canada, Ltd., Montreal 22, Que.
[d] Niagara Brand Chemicals, Burlington, Ont.
[e] Cyanamid of Canada Ltd., Toronto 5, Ont.
[f] Harrisons and Crosfield (Canada) Ltd., Toronto 1, Ont.

looper. On the basis of the 1956–1957 experiments, Phosdrin was recommended to Ontario growers by the Ontario Department of Agriculture and the Canada Department of Agriculture (1958) for control of caterpillars attacking cole crops after the heads have formed.

Malathion, which gave satisfactory control of the imported cabbageworm and the diamondback moth in the 1953–1954 experiments (Harcourt & Cass 1955), apparently does not give appreciable control of the cabbage looper. (In 1955, four applications of 4% malathion dust at 1.25 pounds of toxicant per acre gave 13% control in experimental plots at Merivale, Ont.) Neither does toxaphene control the cabbage looper. Nor does rotenone, recommended for many years for control of caterpillars just before harvest on late cabbage.

The population data in this paper erroneously suggest that the diamondback moth is but a minor pest of cabbage in the Ottawa Valley. It was pointed out in an earlier paper (Harcourt *et al.* 1955) that untreated check plots in fields treated with insecticides do not give a true picture of the relative abundance of the three species; unpublished data indicate that populations of the diamondback moth in check plots of such fields are much lower than those of adjacent untreated areas, apparently because the insecticides repel ovipositing moths.

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NOTICE TO MEMBERS

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Protection of Citrus Pulp Against Insect Infestation with Surface Applications of Pyrethrum-Piperonyl Butoxide Wettable Powder[1]

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ABSTRACT

The seasonal storage of dried citrus pulp has created a serious warehouse insect problem because this commodity is very susceptible to insect infestations. The presence of insects not only affects the composition of the dried citrus pulp but also makes it undesirable to shippers, warehousemen, and the consumer because of the danger of spreading the infestation to other commodities in the vicinity. Being an animal feed it must not contain any harmful insecticide residues and, therefore, the insecticides and the methods of applying them on this commodity are limited. The weekly aerosol applications of synergized pyrethrum commonly used for the past several years have been very expensive and not very effective. The problem has been to develop an effective low cost treatment acceptable under Section 406 of the Food, Drug, and Cosmetic Act.

Laboratory tests showed that wettable powder formulations produced heavier, longer-lasting, and more toxic surface residues than aerosols containing the same active ingredients. This paper describes the evaluation of the practicability and comparative effectiveness of these findings under field conditions. Thirteen stacks of feed were treated with pyrethrum-piperonyl butoxide: 2 with aerosol formulations and 11 with the wettable powder formulations applied to the surface of the stacks. The insecticide residue obtained with wettable powder was more than 10 times that obtained with the aerosol treatment. Some of the insecticide migrated through the burlap bags, but the amount was very small in comparison with the amount that accumulated on the stack surface. The use of vinyl stack covers over the feed after two applications of the wettable powder appears very promising. The wettable powder was much more effective in maintaining a low insect population in the feed than the aerosol treatments. The tests also showed that it is practical to use monthly applications of wettable powders at the rate of 5 mg. of pyrethrum and 50 mg. of piperonyl butoxide per square foot to treat large quantities of stored citrus pulp and that the cost of treatment is less with wettable powders than with aerosols.

Insects pose a very serious problem to the dried-citrus pulp producers. The feed is very susceptible to infestation by a large number of stored-product insects, according to Laudani *et al.* (1956). The most common species attacking this commodity, in the order of their relative importance, are the saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.)), the cigarette beetle (*Lasioderma serricorne* (F.)), the Indian-meal moth (*Plodia interpunctella* (Hbn.)), the almond moth (*Ephestia cautella* (Wlk.)), the Mediterranean flour moth (*Anagasta kühniella* (Zell.)), flour beetles (*Tribolium* spp.), the flat grain beetle (*Laemophloeus minutus* (Oliv.)), and the Angoumois grain moth (*Sitotroga cerealella* (Oliv.)).

Animal feed is considered a processed commodity and as such falls under the jurisdiction of Section 406 of the Food, Drug, and Cosmetic Act. According to law, it should not contain any insecticide residue because no tolerances have been established for such products. However, the necessity for protecting the feed against insect damage has been established, and, therefore, the use of pesticides relatively nontoxic to humans and animals has been unofficially permitted. Synergized pyrethrum in the form of an aerosol has been used almost exclusively for this purpose. Over a period of 3 years the authors have observed that these pyrethrum aerosol treatments have to some degree suppressed the insect infestations but have failed to eliminate or prevent infestation to the satisfaction of the producers and the consumers.

Studies conducted by Kantack & Laudani (1957) on the comparative toxicity of various pyrethrum formulations showed that wettable powder produced quicker action, higher mortalities, and longer-lasting residues than other formulations of the same insecticide. These studies were so promising that it was found advisable to proceed with the evaluation of the wettable powder treatment on a field-test basis.

The investigation described in this paper was arranged to obtain information on the following points:

1. The practicability of using wet sprays on large stacks of stored, bagged feed.
2. The comparative effectiveness of wettable powder and aerosol treatments of pyrethrum-piperonyl butoxide to control or prevent insect infestation in dried-citrus pulp.
3. The most effective rate and frequency of applying synergized pyrethrum wettable powder.
4. The effect of covering synergized pyrethrum-treated stacks with plastic film covers.

PROCEDURE.—*Description of the Test.*—The test was conducted in an all-masonry warehouse containing approximately 50,000 square feet of floor space. The dried citrus pulp was in 8-oz. burlap bags each containing 100 pounds of the feed. The bags were arranged in 13 stacks, the dimensions and positions of which are shown in figure 1. The feed had been in the warehouse for several months and had a natural insect infestation when the test was started in June 1957.

Two formulations of synergized pyrethrum were used in the test. The aerosol formulation contained 0.2% of pyrethrins, 2.0% of piperonyl butoxide, 50% of tetrachloroethylene, and 47.8% of deodorized kerosene by weight. The wettable powder contained 2% of pyrethrins, 20% of piperonyl butoxide, and 78% of inactive ingredients by weight.

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[2] The authors wish to thank the Minute Maid Corporation of Orlando, Florida, for the use of their warehouse and the assistance of their bacteriologist, Mr. D. I. Murdock; the Fairfield Chemical Division, Food Machinery and Chemical Corporation, for the donation of the insecticide used in the test and the assistance of their technical representative, Mr. Carl Watts; and the Bakelite Corporation for the donation of the 8-mil vinyl tarpaulins.

[3] This is one of the field stations of the Stored-Product Insects Section, Biological Sciences Branch, Marketing Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

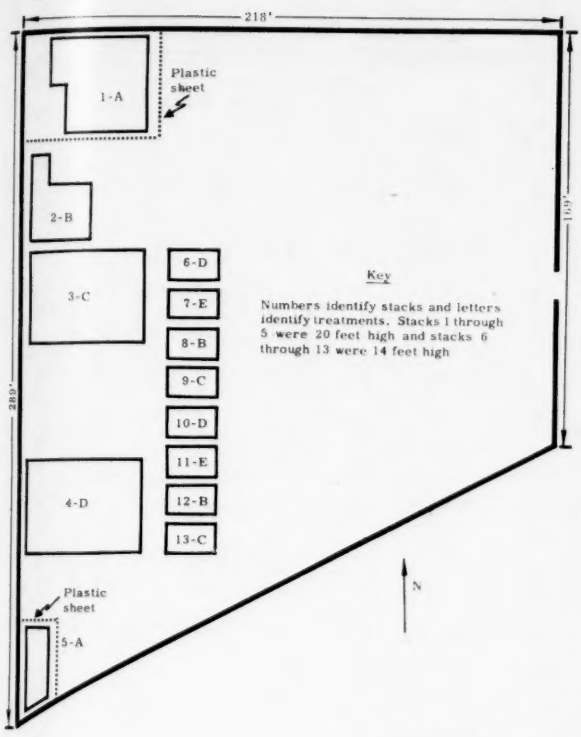

FIG. 1.—Citrus pulp storage warehouse of the Minute Maid Corporation, Plymouth, Florida.

The aerosol formulation (Treatment A) was applied at weekly intervals at the rate of 2½ pints per 10,000 cubic feet of total space. A thermal aerosol generator set to deliver particles of about 5 to 10 microns mass median diameter was used. The area surrounding each of the stacks treated with the aerosol formulation was enclosed with 4-mil polyethylene sheets to insure that all of the insecticide discharged remained in the given area. The amount of insecticide used per stack in four weekly aerosol treatments was equivalent to that used in one monthly application of the pyrethrum-piperonyl butoxide 5–50 mg./sq. ft. wettable powder treatment (Treatment B).

The wettable powder was applied to the surface of the stacks at the rate of 5–50 and 10–100 mg. of pyrethrum-piperonyl butoxide per square foot, respectively. The rate and frequency of application are shown in table 1. The lower rate of application (Treatments B and C) was obtained by adding ½ pound of the wettable powder (2% of pyrethrum+20% of piperonyl butoxide) per gallon of water and applying this mixture at the rate of 1 gallon per 1,000 square feet of exposed bag surface or 2 gallons per 1,000 square feet of actual side and top stack surfaces. The increased surface produced by the curvature of the bags accounts for doubling the volume of spray material used. The 10–100 mg. treatments (Treatments D and E) were obtained by treating the stacks twice with the same mixture used for the lower rate of application.

The equipment used for applying the wettable powder was a 50-gallon power sprayer with a cattle spray gun having a No. 3 circular spray disc. The unit was operated

Table 1.—Rate and frequency of wettable powder treatments.

TREATMENT	RATE OF APPLICATION (MG./SQ. FT.)	FREQUENCY OF APPLICATION					
		Initial	1 Mo.	2 Mo.	3 Mo.	4 Mo.	5 Mo.
A[a]							
B	5–50	X	X	X	X	X	X
C	5–50	X	X	—	X	—	X
D	10–100	X	—	—	X	—	—
E	10–100	X	X[b]	—	—	—	—

[a] Aerosol treatment applied at weekly intervals at the rate of 2½ pints of 0.2–2.0% pyrethrum-piperonyl butoxide formulation per 10,000 cu. ft.
[b] Immediately after the stacks were treated, they were covered with vinyl tarpaulins and then sprayed again.

at a pressure of 300 p.s.i. The spray was applied to the surface of the stacks in an up-and-down sweeping motion to insure good coverage.

Two stacks (Treatment E) received two monthly applications of the wettable powder at the rate of 10–100 mg. of pyrethrum-piperonyl butoxide per square foot and, immediately after the second treatment, the two stacks were covered with 8-mil vinyl tarpaulins. Polyethylene tubes filled with sand were placed on the ends of the tarpaulins to hold them securely to the floor of the warehouse. The outside surfaces of the vinyl tarpaulins were then sprayed with the wettable powder at the rate of 10–100 mg. per square foot.

Sampling.—To determine the insecticidal residue being obtained with the various treatments and the persistency of this residue, burlap patches were pinned on the sides and top of each stack before the treatments were started. A series of seven 1-square-foot patches were placed in a diagonal row from lower left to upper right corner of each side and 28 similar patches were placed at random on the top surface of each stack. Immediately following the initial treatment and at monthly intervals just prior to the treatment, a patch was removed from each side and four from the top of each stack. The patches from the sides were selected to represent four relative stack heights. These patches were chemically analyzed for piperonyl butoxide using a modified Williams & Sweeney (1956) procedure for the separation and the Jones *et al.* (1952) method for the color development.

Samples of the feed for biological determinations and chemical analyses were taken at monthly intervals during the test. A horizontal slit was made on the side of the bag and, with the aid of a specially designed funnel-scoop, quart-size samples of the feed were extracted Every effort was made to take these samples from as close to the surface of the bags as possible. For biological evaluation, 1 quart of feed was taken from a bag selected at random from each of the four sides and 1 quart from each of four bags located on the top of the stack. For chemical analysis a 1-quart sample was taken from two bags located at opposite sides of the stack and two additional samples from bags located at the top of the stack. The above samples were taken monthly just before the insecticide was applied.

RESULTS—The chemical analyses of the patches (table 2) showed that very little residual deposit was obtained with the aerosol applications, whereas a very high deposit was obtained with the wettable powder treatments. It was interesting to compare the residues obtained with the aerosol treatment (A) and the monthly wettable

Table 2.—Piperonyl butoxide residue on burlap patches based on the average of eight samples per stack.

Treatment	Stack No.	Age of Test (in Months) When Samples Were Taken					
		0[a]	1	2	3	4	5
		Mg./Sq. Ft.					
A	1	1.0	1.8	4.9	19.5	—	—
	5	2.1	2.5	7.0	8.1	—	—
	Average	1.5	2.1	5.9	13.8		
B	2	53.5	45.8	129.5	118.8	216.4	—
	8	35.7	43.5	83.1	167.2	270.0	363.9
	12	32.3	35.3	67.7	165.6	293.2	386.3
	Average	40.5	41.5	93.4	150.5	259.8	375.1
C	3	18.0	54.4	98.6	93.6	123.2	—
	9	39.6	38.6	90.2	95.2	222.9	221.1
	13	28.8	33.3	65.7	77.3	229.2	264.3
	Average	28.8	42.1	84.8	88.6	191.7	242.7
D	4	69.3	55.2	67.5	56.9	226.0	—
	6	83.0	77.6	64.9	74.7	231.0	268.7
	10	85.9	70.3	68.1	58.1	203.4	242.6
	Average	79.4	67.7	66.8	63.2	220.1	255.6
E	7	71.3	55.1	—	—	—	195.0
	11	44.9	57.8	—	—	—	139.2
	Average	58.1	56.4				167.1

[a] Immediately after the first application.

Table 3.—Number of insects per quart of citrus pulp based on the average of eight 1-quart samples.

Treatment	Stack No.	Age of Test (in Months) When Samples Were Taken					
		0[a]	1	2	3	4	5
A	1	0.2	0.5	23.3	52.3	—	—
	5	.3	5.5	70.8	—	—	—
	Average	.2	3.0	47.5	52.3		
B	2	0	.7	2.7	8.8	8.3	—
	8	0	.2	3.7	10.5	8.2	6.1
	12	0	.2	3.0	6.3	10.3	.7
	Average	0	.3	3.1	8.5	8.9	3.4
C	3	0	1.0	4.7	12.2	22.8	—
	9	0	.2	5.6	6.6	11.7	4.1
	13	0	.2	.7	8.3	16.0	1.5
	Average	0	.4	3.6	9.0	16.8	2.8
D	4	0	.2	2.3	8.0	17.3	—
	6	0	.2	8.6	6.1	23.1	3.5
	10	0	.3	4.6	7.6	19.2	1.0
	Average	0	.2	5.1	7.2	19.8	2.2
E	7	0	0	—	—	—	0
	11	0	.5	—	—	—	0
	Average	0	.2				0

[a] Samples taken just prior to first application.

powder treatment (B). The amount of insecticide used in the two treatments was exactly the same, yet over a period of 3 months the maximum piperonyl butoxide deposit with the former was only 13.8 mg. per square foot and with the latter 150.5 mg.

Another very important thing shown by these analyses was the persistency of the wettable powder residue. In the 10–100 mg. per square foot treatment (D) the piperonyl butoxide residue by actual analysis immediately after the first application was 79.4 mg. per square foot. With no additional treatments in the 3 months which followed, the chemical analyses showed that there were 67.7, 66.8, and 63.2 mg. of piperonyl butoxide per square foot, respectively. This was a loss of only 16.2 mg. or less than 20% in a period of 3 months. The high rate of deposition and the persistency of synergized pyrethrum wettable powder residue were further shown by the chemical determinations of the 5–50 mg. treatment (C). A high level of deposit (242.7 mg./sq. ft.) was accumulated over a period of 5 months with only three applications.

The biological data further showed the superiority of the wettable powder over the aerosol treatment (table 3). Although no insects were observed in many of the samples taken just prior to the first treatment, the presence of full-grown larvae, pupae, and adults at the 1-month inspection indicated that the pulp was infested when the test was started. The insect count rose rapidly in the aerosol-treated stack to an average high of 52.3 insects per quart sample in 3 months. During the same period the highest insect count in any of the wettable powder-treated stacks was less than 10 insects per quart sample. These data also indicated the interval between treatments should not exceed 1 month. In both cases where the treatments were spread over a period greater than 1 month, the insect population increased sharply (Treatments C and D at 4 months). However, the population of the succeeding generation returned to a low level following each application (Treatments C and D at 5 months).

The chemical analyses of the feed (table 4) showed that some of the insecticide migrated through the burlap and into the pulp with both the wettable powder and aerosol treatments. In proportion to the amount of insecticide found on the surface of the bags, greater migration occurred with the aerosol treatments. However, on the basis of the actual amount of insecticide applied, contamination was greater with the wettable powder treatments. This would be expected since the residue obtained with the wettable powder formulations was greater and more persistent.

The two tarpaulin-covered stacks which had been sprayed twice at monthly intervals with wettable powder at the rate of 10–100 mg. of pyrethrum-piperonyl butoxide per square foot prior to covering (Treatment E) were broken down 4 months later. Large numbers of dead insects covered the top of the stack and the surrounding floor but no live insects were found in any of the samples taken from these two stacks. This was the only treatment which completely eliminated the insect infestation present at the start of the test.

DISCUSSION.—The test described definitely showed that it was practical to use wettable powder sprays on bagged animal feed stored in large lots in warehouses. With the equipment described, all surfaces of the stacks were thoroughly treated without difficulty even though some of the aisles between the stacks were less than 3 feet wide. The volume of spray used did not wet the stack surfaces but just moistened them. In less than 30 minutes the spray could not be detected by feel. The wettable powder residue was only slightly visible on the sprayed

Table 4.—Piperonyl butoxide residue in citrus pulp based on the average of four samples.

TREATMENT	STACK NO.	AGE OF TEST (IN MONTHS) WHEN SAMPLES WERE TAKEN (P.P.M.)					
		0[a]	1	2	3	4	5
A	1	3.1	5.5	5.3	4.9	—	—
	5	3.8	4.2	5.1	5.5	—	—
	Average	3.4	4.8	5.2	5.2		
B	2	5.6	5.2	8.1	11.3	9.4	—
	8	5.8	5.7	8.0	9.9	8.3	9.5
	12	4.4	6.4	7.8	7.4	7.7	10.8
	Average	5.2	5.7	7.9	9.5	8.4	10.1
C	3	5.0	4.8	5.7	7.4	6.5	—
	9	5.9	6.0	8.0	7.6	15.3	6.0
	13	5.7	5.8	8.4	7.4	7.0	4.8
	Average	5.5	5.5	7.3	7.4	9.6	5.4
D	4	4.5	6.0	5.7	6.8	9.1	—
	6	6.2	8.1	6.1	6.4	7.0	14.8
	10	7.1	7.0	7.0	10.1	7.3	7.1
	Average	5.9	7.0	6.2	7.7	7.8	10.9
E	7	—	7.0	—	—	—	6.8
	11	—	6.9	—	—	—	6.4
	Average		6.9				6.6

[a] Samples taken just after first application.

surface of the bags. As soon as the bags were handled, the residue usually disappeared to where it could not be detected visually.

Although sufficient replications were not available for statistical analysis of the data, the results were so conclusive that it could be definitely stated that wettable powder spray treatments were far superior to the aerosol treatments in their effectiveness to suppress insect infestations. The residue obtained with the wettable powders was considerably heavier and much more persistent.

The results indicated that the pyrethrum-piperonyl butoxide wettable powder treatment applied monthly to the sides and top of stacks at the rate of 5–50 mg. per square foot was the most effective treatment tested.

The use of plastic film tarpaulins for covering stacks of feed treated with a surface residual spray appeared very promising. The tarpaulin apparently supplemented the effectiveness of the treatment by mechanically excluding any infestation from without and confining the inside infestation to the treated surface. The potential of such a treatment, spraying the surface of a stack with a residual then covering with a plastic tarpaulin for long-term storage, is tremendous for long-term storage. It cuts down on the amount of insecticide used, on labor involved, and provides a treatment with the least possible contamination of the commodity. However, this phase of the test should be considered as purely of an exploratory nature. Additional testing must be done before firm conclusions can be drawn.

A very important finding was the small amount of insecticide that migrated to the commodity. The average amount of piperonyl butoxide in the pulp from any of the treatments was less than 11 p.p.m. If the ratio of the pyrethrum to piperonyl butoxide remained constant at 1:10, this would mean that there was less than 1 p.p.m. of pyrethrum. Furthermore, since the samples were obtained close to the surface of the bags, judging from other residue studies, a composite sample would have considerably less insecticide. The tolerance set for pyrethrum and piperonyl butoxide on corn for post-harvest application is 3 and 20 p.p.m., respectively (Anonymous (1957)). Since corn is used for animal feed and can contain the amount of these two insecticides specified above, the possibilities of approval for citrus pulp feed at the same or lower levels should be good.

The insect problems and the methods of storing many of the bagged animal feeds and raw commodities are basically the same. The information obtained from these tests could, therefore, be applicable to the protection of all bagged animal feeds; bagged seeds such as corn, sorghum, and cotton; dried peas and beans; rough rice; and other raw commodities.

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Seasonal Distribution of Mites on Texas Grapefruit[1]

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Abstract

The important species of mites on grapefruit in Texas were found to fluctuate considerably during the year. *Eutetranychus banksi* (McG.) was found to have its greatest increase in population during May–July. Increases were associated with periods of very little rainfall, lower relative humidity and 8 to 10 hours per day of average temperatures above 80° F. Rainfall appeared to be a regulatory factor. Populations of the citrus rust mite, *Phyllocoptruta oleivora* (Ashm.), increased particularly during periods of high relative humidity while periods of low relative humidity and very windy weather seemed unfavorable. Rust mites increased generally following periods of greater precipitation, and increases in winter occurred each year.

Typhlodromus spp. increased during May and numbers were greater during the summer and fall months. Tydeid and *Brevipalpus* mites were more numerous during the latter half of the year.

Very little information has been available regarding the seasonal occurrence of the mites on citrus in the Lower Rio Grande Valley area. Most growers in this area apply sulphur dust directed primarily against the citrus rust mite, *Phyllocoptruta oleivora* (Ashm.), for their seasonal pest control program. Following a post-bloom application of sulphur, many growers apply again in May and August or as infestations of the rust mite justify, while a few growers make applications on a 45-day schedule. Some growers expected populations of citrus rust mites to increase following periods of rain. The tetranychid mite, *Eutetranychus banksi* (McG.), has not been considered a pest of economic importance by many growers while some have relied on sulphur for its control. Other growers have found *banksi* difficult to control with sulphur. Investigations were begun in January 1954 to determine the seasonal population trends of the various mite species on grapefruit leaves and the factors that may influence changes in populations in order to establish a better basis to attain mite control.

Several authors have reported that climatic factors influence populations of citrus red mite, *Panonychus citri* (McG.), on citrus. Boyce (1936) reported that under normal conditions in California the peak infestations of this mite usually occur in the spring, from April through June, and are frequently important in the fall, from October to December. During exceptionally warm winters, heavy mite infestations may exist from fall throughout the entire winter and spring. Woglum (1939) reported that during the week of September 16 to 23 when temperatures reached 107 to 113° F., active citrus red mites were almost all destroyed while egg mortality appeared less than that of the crawling spider mites. De Bach *et al.* (1950) reported that citrus red mite populations tended to increase during the fall and winter, although a winter reduction in some groves resulted in a low population about February. The spring peak occurred about May or June which was in turn followed by lower populations. Jeppson *et al.* (1957) reported that low humidity and moderate to high temperatures were unfavorable for development of the citrus red mite in California on lemons. The rapid increase in mite populations after the middle of September indicated that a period of high temperatures without low humidity had but limited adverse effect on mite populations when such conditions occurred during the early part of a new growth cycle. Thompson & Griffiths (1950) stated that *P. citri* is often more numerous during and following periods of dry weather in Florida. Pratt (1955) in Florida, concluded that severe outbreaks of *P. citri* in the spring can only occur when the preceding December is cold and spring rainfall is below normal. He further reported that infestations in the summer and fall are influenced primarily by the amount of rainfall in those seasons.

Other authors have reported that spider mite populations fluctuate under various conditions. Jeppson *et al.* (1953) stated that orange leaves of each growth cycle that were not fully mature appeared to be more favorable than mature leaves for development of citrus red mites, and that the maximum amount of this stage of growth occurs during spring and fall in California and is correlated with highest infestations of the mite. Henderson & Holloway (1942) reported the number of citrus red mite eggs produced on young and medium-age leaves were significantly greater than on old leaves and the feeding injury influenced egg production to a much greater extent on flush-growth leaves than on older leaves. They attributed excessive leaf injury by the citrus red mite as a factor capable of causing a reduction in population since populations often decrease rapidly when climatic conditions are apparently favorable for development and natural enemies are extremely scarce. Fleschner *et al.* (1956) found citrus red mite females (from heavily-infested leaves) would spin down from the leaf and a gentle breeze would carry them to other trees. Huffaker & Spitzer (1950) showed in greenhouse tests with pears that strong wind was an inhibiting factor in the early development of two-spotted mite, *Tetranychus telarius* (L.). Boudreaux (1958) reported that several species of tetranychid mites on cotton produced fewer eggs at a lower rate with a shorter life-span of egg-laying females under conditions of constant high relative humidity. Also, indications were that a higher rate of feeding occurred with *T. telarius* under conditions of lower relative humidity. Kremer (1956) reported *Bryobia praetiosa* Koch on deciduous fruit leaves in Germany as a species which displays a liking for dry conditions. He also reported that heavy rainfall eradicated the post-embryonic stages while affecting egg populations very little.

Several factors that adversely affect rust mite populations have been reported. It was reported by Yothers

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Table 1.—**Monthly populations of *Eutetranychus banksi* (McG.) on grapefruit at Weslaco, Texas.**

	1954				1955				1956			
	Untreated		Sulphur[a]		Untreated		Sulphur		Untreated		Sulphur	
MONTH	MPL[b]	EPL[b]	MPL	EPL	MPL	EPL	MPL	EPL	MPL	EPL	MPL	EPL
January	1.0	2.1	2.8	1.1	3.6	1.8	31.3	9.5	5.1	6.3	18.0	19.8
February	.3	—[c]	2.0	2.6	2.2	2.0	9.7	3.8	.9	.6	8.5	6.2
March	2.4	.1	.4	.3	3.5	3.2	2.2	2.3	.2	.2	.3	.1
April	.8	.8	1.6	.6	2.9	3.4	—[c]	.1	.3	.4	.1	.3
May	14.5	11.8	.8	.4	100.9	40.2	1.2	3.0	5.1	8.5	2.8	5.9
June	20.1	5.3	49.2	30.8	29.8	28.7	2.3	1.4	59.0	43.3	6.0	2.5
July	.03	.1	6.4	7.0	6.4	5.5	54.3	47.5	12.3	11.1	16.2	11.5
August	1.7	3.0	1.6	.9	4.6	5.0	17.6	21.5	7.1	5.9	28.6	41.5
September	2.0	1.7	1.9	1.5	2.0	1.8	.5	.7	19.9	20.0	28.2	33.4
October	.5	.2	2.3	1.5	1.8	.9	3.1	2.2	21.1	16.6	24.9	24.1
November	.8	.4	5.7	2.0	3.0	3.9	4.0	5.6	26.6	13.7	18.1	17.0
December	2.8	1.5	21.5	14.4	8.2	8.8	11.6	7.9	12.1	5.9	7.7	7.2

[a] Sulphur applications as follows: 4/23, 7/24, 9/23 (rained off in 22 hours) and 10/20/1954 (rained off in 74 hours); 3/19, 5/21 and 8/22/55; 2/26, 5/19 and 9/4/5 (rained off in 3 hours).
[b] MPL, mites per leaf; EPL, eggs per leaf.
[c] Present, but too few for average figure.

(1917) that a sudden severe cold spell was fatal to rust mites, not only the adults and young but also the eggs were destroyed. Watson (1932) reported rust mite populations increased most rapidly during dry weather in Florida. Yothers & Mason (1930) reported that freezing weather and seasons of dry weather adversely affected rust mite populations whereas hot sunshine and rains seemed only to drive them to the more protected surfaces of the fruit and leaves. Injurious numbers suddenly decreased a short time after the beginning of the summer rains, probably because of an entomogenous fungus.

Dean (1952) found no authoritative record of *P. citri* from Texas. Recently, however, citrus red mites were observed on two backyard citrus trees in the Houston area. Some of the same conditions that have been reported to cause fluctuations in populations of citrus red mite and other mites in other areas have also been found important in explaining fluctuations of mite populations on citrus in Texas.

METHODS.—Leaf samples were taken 2 to 7 feet from the ground around the tree. The third leaf from the apical end of the terminal flush of growth was selected, varying to the second or fourth leaf as necessary. Ten leaves were selected in each quadrant for a total of 40 leaves from each of five trees for a composite sample of 200 leaves. In February 1955, leaves were sampled from two additional untreated trees. The various phytophagous and beneficial mites on the foliage were counted under 18× magnification of a stereoscopic microscope. The monthly populations of *banksi* and citrus rust mites are shown in tables 1 and 2, respectively.

Sulphur dust was applied to the east and to the west sides of the 18- to 23-year-old pink grapefruit trees by a Model ZA Root power duster which was mounted on a small trailer and pulled through the grove by a tractor. The dosage was slightly more than 1 pound per tree. Weather conditions were nearly ideal at the time of each application. Special care was taken in applying from both sides of the trees to insure maximum coverage.

Weather instruments were at the grove site. The record of "miles of wind per day" was derived by dividing the total monthly wind movement, as recorded by an anemometer, by the number of days in the month.

RESULTS AND DISCUSSION.—The various mite populations are not discussed in the order of the importance of the species in this area. Weather conditions were found to be closely associated with fluctuations of certain mite populations, many times accounting for the changes in populations of certain species.

In each of the 3 years of this study weather conditions varied considerably from normal based on 43-year rainfall, 28-year wind, and 32-year relative humidity. However, an opportunity was afforded to study the various mites under extremely variable weather conditions.

Eutetranychus banksi.—This species and its eggs were found in every month of the year as shown in table 1. The egg population closely paralleled that of the mite population. Lowest populations occurred during February to late April. The largest populations were found during May and June on the untreated trees and occurred a month or two later on the trees treated with sulphur for control of the rust mite. A much smaller population was found during December and January although higher at that time, on the sulphur-treated trees. *Stethorus atomus* Casey

Table 2.—**Monthly populations of the citrus rust mite on grapefruit at Weslaco, Texas.**

	MITES PER LEAF					
	1954		1955		1956	
MONTH	Untreated	Sulphur[a]	Untreated	Sulphur[a]	Untreated	Sulphur[a]
January	0.1	1.4	62.2	29.6	755.8	1001.3
February	.1	9.0	12.9	34.1	313.0	700.0
March	.1	.7	25.5	69.4	44.4	.5
April	.4	3.2	1.4	—[b]	38.4	.4
May	15.9	.2	9.6	.1	337.1	8.3
June	101.2	4.1	9.7	—[b]	835.0	—[b]
July	47.0	7.0	18.4	—[b]	463.8	1.2
August	150.1	.4	44.3	—[b]	132.4	6.1
September	471.9	8.5	105.0	—[b]	105.4	1.3
October	529.7	2.9	257.2	2.4	250.8	13.2
November	610.0	.8	814.6	37.4	222.4	67.0
December	208.8	3.9	806.2	214.9	33.4	62.3

[a] Sulphur applications as follows: 4/23, 7/24, 9/23 (rained off in 22 hours) and 10/20/1954 (rained off in 74 hours); 3/19, 5/21 and 8/22/55; 2/26, 5/19 and 9/4/56 (rained off in 3 hours).
[b] Numbers too few for average figure.

and *Chrysopa* spp. have been noted as important predators of this mite during the latter period. Populations of *banksi* were found to build up on the trees next to a dusty road before general increases were noted throughout the rest of the grove.

The peak mite population occurred in June 1954 while the peak egg population was in May (the only time the egg peak was observed to precede the mite peak). During April, 6.85 inches (5.49 inches above normal) of rain fell and apparently delayed the buildup of mites during May and June. Hurricane rains that fell in June totaled 4.45 inches during a 3-day period and further reduced this potential increase. A count on the treated trees immediately following these rains indicated a reduction from 49.2 to 4.3 mites per leaf and from 30.8 to 2.1 eggs per leaf. Individuals of a heavy *banksi* population will migrate from the preferred upper surface to the lower surface, and during rains it seems apparent that a smaller percentage of the total mite population will be destroyed than when a lighter population is confined entirely to the upper surface of the leaves. Although July had 1.63 inches below normal rainfall, August and September had 0.99 and 3.58 inches, respectively, above normal rainfall. The application of sulphur in July 1954, together with the rather heavy rain in August-September, were apparently important factors responsible for the lower *banksi* population during this period. However, rains fell during 1954 at periods somewhat less favorable for higher populations to develop.

The peak of population of *banksi* and its eggs came early in May 1955. The winter carryover was larger than for the other 2 years. Recorded temperatures and humidities were not available until August 1954. However, in 1955 there were 2.9 and 1.8 more hours per day above 80° F., and 0.32 and 1.5 more hours per day above 90° F. in April and May, respectively, than in the same months of 1956. At least 14% more wind movement occurred during February–July, 1955 than in the same period in 1954 and 1956. Combined rainfall for April and May totalled 7.33, 0.43 and 1.82 inches for 1954, 1955 and 1956, respectively. July, August and September had 0.83, 2.08 and 6.33 inches above normal rainfall and mites and their eggs were progressively less numerous during late summer. A small increase was then found during the last 3 months of the year with subnormal rainfall during that period. This increase was more pronounced on the sulphur-treated trees.

The highest population of *banksi* during 1956 occurred in June. Only 0.03 inch of rain fell in January. The following 3 months (with sub-normal rain), had 1.22 inches more than in 1955. Rainfall during the remainder of the year was more than an inch per month below normal, excepting June and November which had 0.92 and 0.96 inches, respectively, below normal. Populations were very heavy during the last 6 months of the year which resulted in a higher yearly total than experienced in the two previous years. The calm weather, slightly above 80° F., that followed the applications of sulphur in February and April appeared to have contributed to the lower populations on the treated trees during the first 6 months of the year.

During August-December of 1954, 1955 and 1956, there were 869 and 190, 879 and 145, and 1204 and 362 hours above 80° and 90° F., respectively. During July-December of 1955 and 1956, there were 1206 and 193 and 1600 and 530 hours above 80° and 90° F., respectively. Average mean relative humidity was more than 12% less in July-December of 1956 than in the same period of 1954 or 1955. The warmer and drier weather that prevailed during the last 6 months of 1956 as compared with 1954 and 1955 apparently favored the larger populations of *banksi*.

The months of greatest wind movement were March, April and May with noticeably less wind in June. September had the least wind and usually had the most rain. A high relative humidity occurred even though wind movement was high. The relative humidity averaged, from 4.9 to 10 hours per day, below 50% with several low readings below 20 and even down to 8%.

The yearly total population was therefore less when rainfall assisted in reducing populations during the period of greatest development potential (May-June) and greater when hot and dry weather occurred during or following that period. Rainfall was subnormal for the month of May in the following amounts: 2.54 in 1954, 2.79 in 1955, and 2.31 inches in 1956. Average rainfall during May seemed to suppress the potential increase. The yearly rainfall was 26.23 inches for 1954, 21.49 for 1955 and 7.81 for 1956.

Certain other factors that may be related to changes in mite pouplations may justify investigation. Jones & Steinacker (1957) in California, and Martin (1942) in Arizona, reported that the sugar content of the citrus leaf was highest during the winter months preceding the start of spring growth. These authors further reported a large increase in starch in the leaves just prior to the spring flush of growth. Winter increases of *banksi* appeared to be associated with high sugar content of the leaves, but such winter increases were not nearly as great as the increases during May-July when the average temperatures for 8 to 10 hours per day were above 80° F. and apparently more favorable for development. The high starch content of the leaves seemed to occur during the period when populations of *banksi* were lowest.

Citrus rust mite.—Rust mites did not show as clear a population pattern as *banksi*, as shown in table 2. Abnormal weather conditions that prevailed during 1955 and 1956 were considered factors particularly affecting such fluctuations in population. The lowest population occurred in April with the next lowest in March. Rust mite eggs were particularly noticeable during September (the month with the least wind and usually with the greatest rainfall) and during the winter increase period in 1955 and 1956. Fruit injured by the rust mite was observed as early as in late May. Infestations were found each winter during the 3-year period under study.

Relative humidity appeared to be the most important weather factor influencing citrus rust mite populations, except extremely cold temperatures which seldom occurred during the period of this study. Temperatures, as recorded by a hygrothermograph, were above 100° F. once in March and once in July of 1954 and in April 1955. Record of 28°, 31° or 32° F. were noted only once during the study and these readings occurred only for a very short time. Populations increased when relative humidity did not fall below 50 to 60% during the day and rose to 95 to

100% during the late afternoon and at night.

Populations increased more following above-normal rainfall and when the wind averaged 40 to 55 miles per day during the month, together with continued high relative humidity. Populations increased under conditions of very little rainfall, 74 to 85 miles of wind per day, relative humidity dropping twice to the 10 to 20% range but with high relative humidity the remainder of the month. Still greater increases occurred when the same conditions prevailed (no low relative humidities were recorded) as in June-July 1955 and in May-June 1956. A pronounced increase in population occurred in October-November 1955 with one-third normal rainfall for the period, 42 miles per day of wind and high relative humidity except for three rather short periods of 15 to 20% and six short periods of 20 to 30% relative humidity.

The greatest increase of citrus rust mites observed during the study was in the winter of 1955–56, when a sharp increase was noted from November through January while the average relative humidity was above 70% and winds were relatively light except in November. Relative humidity dropped below 20% four times for very short periods but was otherwise very high while rainfall was subnormal. According to the U. S. Weather Bureau in Brownsville, Texas, only two conditions differed strikingly for the period. Relative humidity was 15% higher than normal during the period and 16 foggy days occurred in December. Fog held on until late morning for several days.

Certain weather factors were found to be associated with decreasing citrus rust mite populations. Low humidity readings in the 20 to 30% range for some duration were followed by lower rust mite populations although one or two such humidity readings with high relative humidity the remainder of the month would usually affect the results of the next population count very little. The most drastic reduction in population came in March-April 1955 when the relative humidity dropped to 8% on one occasion and below 20% twice. During the latter period, there were 12 hours below 20% and 60 hours below 30% in addition to 105 miles of wind per day (the windiest period during the study).

The general averages showed that citrus rust mite populations were lower during March and April. Although greater wind movement during this period was closely associated with lower rust mite populations, only a general deduction can be made. Monthly averages of miles of wind per day of 85 to 105 seemed unfavorable, whereas, those of below 50 seemed most favorable. for increased populations during the 3-year period.

Citrus rust mite populations generally increased following periods of greatest rainfall. This was more pronounced where several showers fell during the month rather than when the bulk of the monthly rainfall occurred on 1 to 3 days, as commonly occurred during this study. Rainfall during the 3-year study appeared to be associated with higher relative humidity and in turn with higher rust mite populations.

Trees were dusted with sulphur during 1954 as mite infestations increased. Rust mite populations were low at the outset of the test and, therefore, the treatment was delayed until April 23. Treatments were applied following the increase in populations in July and September, the latter being washed off after 22 hours. Treatment following the October count, although washed off by rain after 74 hours, apparently helped to hold down winter populations. In the subsequent years, applications were made in late March, May and August except that a late February application was made in 1956 owing to the high populations present. Rather high populations developed every year during the winter. According to the records, September had more rain than any other month, with May second. The possibility of increased populations of the citrus rust mite during or following May, in years of normal rainfall in May should not be overlooked. The high relative humidity, low wind velocity and rainy conditions that occurred during September were accompanied by an increase in rust mites and indicated the possible need for another application of sulphur during October.

Citrus rust mites were effectively controlled after each application of sulphur, however, large populations developed within 2 months if weather conditions were favorable. Very high populations were brought under control when weather conditions were favorable. It was necessary to follow the Weather Bureau forecast closely for calm early-morning periods during which applications of sulphur could be made.

OTHER MITES.—The *Typhlodromus* (Phytoseiidae) populations noticeably increased during May with higher numbers usually found during the summer and fall months. Numbers were lower in the drier year of 1956. Populations of these mites were at their lowest level during March and April on the sulphur-treated trees with the peak usually occurring in July. A reduction in population was then observed during the last 2 months of the year. There were indications that heavy April rains were followed by a lower count in May. However, the largest populations were recorded during the year of greatest rainfall. *Typhodromus* sp., possibly *T. mesembrinus* Dean, was the most common species. *T. floridanus* Muma and *T. conspicuus* (Garman) were found from time to time. Another *Typholodromus* sp., undescribed, appeared to be more closely associated with scale insects than mites.

Tydeid (Tydeidae) mites were more numerous during June-December. Considerably more of these mites were found during the dry year of 1956 than in the 2 previous years. *Pronematus ubiquitus* (McG.) was by far the most numerous of the tydeid mites observed, although *Tydeus* sp. was taken on several occasions. Populations were very low on the sulphur-treated trees.

Other predaceous mites were found to be associated with phytophagous mite populations. *Allothrombium* sp. (Trombidiidae) was taken on occasions and observed to feed on *E. banksi* and its eggs. Adults of this predaceous mite appeared more commonly during May and in October and November, *Balaustium* sp. (Erythraeidae) was taken on one occasion and observed to greedily feed on citrus rust mites. *Anystis* sp., possibly *agilis* (Banks) (Anystidae) was occasionally found in very small numbers.

Other phytophagous mites were of less importance. *Brevipalpus australis* (T.) (Tenuipalpidae) was much more common than *B. phoenicis* (Geijskes). These mites were more numerous on the leaves during the latter 6 months of the year. Generally, fewer mites were recorded on the leaves in April than at other times of the year. A

far greater number was recorded in the drier year of 1956. Few were found on the sulphur-treated trees.

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Methoxychlor Residues on Vegetable Crops[1]

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ABSTRACT

Methoxychlor was applied to vegetables in small field plots at Beltsville, Md., at 1¾ pounds per acre in dusts and in wettable powder and emulsion sprays, and also at 3 or 3½ pounds per acre in wettable-powder sprays. Residues determined at intervals appeared to justify a waiting period of only 1 day from last application to harvest, for summer and winter squash, cantaloupe, eggplant, pumpkin, peppers, tomatoes, Kohlrabi, turnip roots, and strawberries, at the present tolerance of 14 p.p.m. For okra a 1-day waiting period is needed, which would require a tolerance of 20 p.p.m., and for green onions 3 days, which would require a tolerance of 40 p.p.m.

Experiments were conducted in 1955–57 at the Agricultural Research Center, Beltsville, Md., to determine the amount of methoxychlor deposited on certain vegetable crops from spray and dust applications and the subsequent persistence of the residues. Because of the need for harvesting such crops as okra, summer squash, tomatoes, and strawberries several times a week, methoxychlor should be applied up to 1 day before a harvest if full use is to be made of this insecticide.

Methoxychlor was applied to crops in small field plots at 1¾ pounds per acre in a dust and in wettable-powder and emulsion sprays, and also at 3 or 3½ pounds per acre in wettable-powder sprays. Measured quantities were applied to a given length of row. Rotary hand dusters and compressed-air sprayers were used. The sprays were applied with a pressure of approximately 60 pounds per square inch at the rate of 100 gallons per acre. One sweep of the nuzzle was made to each side and top of the row in separate operations, and any remaining insecticide of the measured quantity was applied to the top of the row as evenly as possible. One application was made.

Samples consisting of approximately 500 grams of the edible portions of the vegetables were harvested as soon as the sprays dried, or within 1 hour after treatment, to determine the original deposit. Samples for residues were taken after 1, 3, 7, and 14 days and at weekly intervals thereafter if it was believed that the residue might have persisted longer. On many crops samples were taken on the day of application and washed in water to determine the benefit of washing in the reduction of residues. Residues were determined by the total-chlorine method.

The results of tests on all except the leafy vegetables are presented in table 1. The studies on these have not yet been completed. There was considerable variation in the results. The cause of this variation is not fully understood, but replications of the tests would have been desirable. However, certain conclusions may be drawn.

The deposits of methoxychlor were, in general, low and

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Table 1.—Residues of methoxychlor on vegetable crops.

Date of Treatment	Interval Between Treatment and Harvest		Residues (p.p.m.)			
			1¾ Pounds per Acre			3 or 3½ Pounds per Acre[a]
	Days	Rainfall (in.)	Dust	Wettable-powder Spray	Emulsion Spray	Wettable-powder Spray
			Green onions			
June 12, 1956	0	0	14.6	13.8	—	*23.0*
	1	0	11.1	18.9	—	*15.9*
	3	0	8.7	4.2	—	*10.5*
	8	1.92	1.2	1.1	—	*1.5*
June 4, 1957	0	0	12.1	27.4	60.9	58.9
June 10, 1957	0	0	9.0	23.8	59.0	63.1
	3	0	—	4.6	26.7	26.2
	7	0.11	—	28.7	40.2	40.9
	14	.82	0.4	4.1	4.5	7.9
	0	0	4.8[b]	5.6[b]	70.2[b]	34.6[b]
			Green beans[c]			
Oct. 5, 1955	1	—	—	5.5	—	*16.2*
	5	1.00	—	2.9	—	*7.9*
Sept. 14, 1956	0	0	33.1	20.9	—	*50.4*
	3	0	5.3	7.8	—	*10.5*
	7	0	3.9	6.8	—	—
	10	0.28	1.0	1.9	—	*10.8*
Sept. 2, 1957	0	0	1.5	4.4	—	14.6
	1	0	1.2	4.2	—	10.5
	3	0	1.1	5.7	—	9.9
			Summer squash			
July 9, 1957	0	0	0	8.4	2.9	7.1
	1	0	16.0	5.0	1.1	2.0
	3	0.30	0.	0	1.0	0
	7	.90	0	0	0	0
	0	0	0	0.8[b]	1.8[b]	1.8[b]
			Okra			
Sept. 13, 1955	0	0	0	—	—	3.0
	2	0	0.3	—	—	1.3
	9	0	1.0	—	—	0.5
	14	0	0	—	—	0
Sept. 5, 1956	0	0	4.3	9.1	—	*8.4*
	1	0.28	5.8	7.6	—	*3.2*
	3	.28	1.0	4.4	—	*1.6*
	7	.28	0.4	1.6	—	*3.8*
Aug. 6, 1957	0	0	6.2	9.5	13.2	16.2
	1	0	12.2	19.4	4.8	12.7
	3	0	19.4	10.0	5.2	8.8
	0	0	1.4[b]	0.2[b]	—	—
			Cantaloupe			
Aug. 20, 1957	0	0	1.8	2.7	3.1	2.3
	1	0	1.3	2.5	4.2	1.3
	3	0	0.8	3.0	2.9	2.5
			Eggplant			
Aug. 19, 1957	0	0	8.2	4.5	5.1	1.7
	1	0.05	0	0	0	0
	3	.05	0	0	0	0
	0	0	0[b]	0[b]	0[b]	0[b]
			Pumpkin			
Aug. 13, 1957	0	0	0.4	2.4	0.9	0.8
	1	0	.9	.9	1.2	1.1
	3	0	.9	1.8	1.4	2.3
			Bell peppers			
July 28, 1957	0	0	5.4	1.1	0.9	4.6
	1	0	0.6	3.7	.5	3.4
	3	0	0	0	1.0	0
	7	0	0	0	0	0
			Tomato			
Sept. 10, 1956	0	0	2.3	0.5	—	*0.7*
	1	0	1.1	.3	—	*1.0*
	3	0	0.7	.8	—	*0.9*
	7	0.44	.6	0	—	*.1*
Aug. 12, 1957	0	0	3.0	4.1	2.5	3.1
	1	0	1.1	0.7	—	1.8
	0	0	1.0[b]	1.0[b]	1.7[b]	1.6[b]

[a] Figures in italics for a dosage of 3 pounds per acre.
[b] Samples washed in tap water for 1 minute.
[c] Analyses in 1957 made by the E. I. du Pont de Nemours & Co.
[d] Some evaporation of strip solution causing high residue.

Table 1.—(*Continued*)

Date of Treatment	Interval Between Treatment and Harvest		Residues (p.p.m.)			
			1¾ Pounds per Acre			3 or 3½ Pounds per Acre[a]
	Days	Rainfall (in.)	Dust	Wettable-powder Spray	Emulsion Spray	Wettable-powder Spray
			Winter squash			
Aug. 20, 1957	0	0	2.3	2.7	5.1	4.5
	0	0	0	—	1.5	0
			Cucumber (slicing)[c]			
July 22, 1957	0	0	1.1	2.9	—	2.1
			Cucumber (pickling)			
July 29, 1957	1	0	1.1	1.7	—	1.2
	3	0	0.8	1.1	—	1.0
			Kohlrabi			
June 4, 1957	0	0	1.2	2.6	5.9	5.5
June 10, 1957	0	0	0.2	1.7	2.5	3.7
	1	0	1.4	1.7	2.1	1.2
	3	T	11.4	5.6	2.3	2.5
	7	0.11	2.6	—	2.5	5.1
	0	0	0.8[b]	2.1[b]	2.1[b]	4.1[b]
			Turnip roots			
July 8, 1957	0	0	1.0	0	0.4	0
	1	0.08	0	0.03	.5	0.8
	3	.08	0.3	0	.1	1.8
	7	.98	0	0.2	.6	—
			Strawberry			
May 28, 1957	0	0	1.0	1.8	1.2	—
	1	0	0.4	5.5	6.1[d]	10.9[d]
	0	0	.8[b]	1.1[b]	3.2[b]	2.6[b]

except at the higher dosages below 14 p.p.m., the tolerance level established for most vegetables by the Food and Drug Administration. Residues were higher on small vegetables, such as green onions and green beans, than on squash, pumpkin, and cantaloupe.

Deposits left by 3- or 3½-pound dosages of wettable-powder sprays were little higher, and in many cases lower, than those from 1¾ pounds of either sprays or dusts. The reduction of residues with the higher dosage was generally as rapid as with the lower.

Residues from the emulsion sprays at 1¾ pounds per acre were generally higher than from dusts and wettable-powder sprays at the same dosage. Also residues left by wettable-powder sprays were higher than those by the dusts. Therefore, residues from dusts were below the tolerance level in less time than those from the sprays.

Washing vegetables immediately after the insecticide was applied reduced the residues. However, high residues remained on green onions from the emulsion spray and the higher dosage of wettable-powder spray. No benefit is obtained from washing most of the other vegetables, owing to the low level of the deposit. Residues from emulsion sprays were not as easy to remove by washing as those from the dusts and wettable-powder sprays.

It is believed that the data presented in this report justify a waiting period of 1 day from last application to harvest, for summer and winter squash, cantaloupe, eggplant, pumpkin, peppers, tomatoes, kohlrabi, turnip roots and strawberries, at the present tolerance of 14 p.p.m. For okra a 1-day waiting period is needed, which would require a tolerance of 20 p.p.m., and for green onions 3 days, which would require a tolerance of 40 p.p.m. There is now no tolerance for methoxychlor on either of these crops.

Laboratory Evaluation of Various Residual Sprays for the Control of Dermestids Infesting Milk-Drying Plants[1]

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Abstract

A number of chemicals were evaluated under laboratory conditions for their effectiveness against larvae of the larger cabinet beetle (*Trogoderma inclusum* (Lec.)) and the black carpet beetle (*Attagenus piceus* (Oliv.)). The chemicals were applied as acetone solutions to masonite panels. Ten larvae were exposed to the panels 1 hour after treatment and again later to those panels that had appeared effective during the preliminary test and had aged for various periods of time. Malathion, Chlorthion, Diazinon, and lindane appeared to be the most effective insecticides evaluated to date for use against these dermestids.

In recent years contamination of nonfat dry milk by the larger cabinet beetle (*Trogoderma inclusum* (Lec.)) and to a lesser extent by the black carpet beetle (*Attagenus piceus* (Oliv.)) has become a serious problem for the dairy industry. The insects in nonfat dry milk render the product unfit for human consumption and, if the milk is being sold to the government under the price support program, render the producer ineligible to make further sales until his plant is inspected and found to be free of insects. Increased production and the storage of surpluses for long periods of time have aggravated the existing problems in dairy-product storage and have emphasized the need for research on the prevention of insect contamination. Proper sanitation and the use of residual sprays appear to be the most promising procedures for materially reducing or solving the problem. In these studies a number of chemicals have been evaluated for their effectiveness as residual sprays against the primary insect pests of nonfat dry milk.

Materials and Methods.—Masonite panels, 6″×6″, were treated by means of a pipette with an acetone solution of the chemical to be evaluated. With the exception of pyrethrins and allethrin, all insecticides were applied at the rate of 50 and 200 mg./sq.ft. In addition, malathion was also evaluated at 100 mg./sq.ft. and methoxychlor and lindane at 25 mg./sq.ft. Pyrethrins and allethrin were tested only at 5 and 10 mg./sq.ft. in combination with piperonyl butoxide at 50 and 100 mg./sq.ft., respectively. Piperonyl butoxide was also evaluated as a synergist for malathion, and Arochlor 5460 (a chlorinated biphenyl) was tested as an extender for lindane. The primary evaluation tests were conducted with *T. inclusum* and only those which appeared to be the most effective against this species were evaluated against the black carpet beetle.

Ten larvae of the test insect were exposed to the panels 1 hour after treatment and confined continuously to the treated surface for 6 to 7 days by means of open glass rings. The percentage of dead and moribund larvae was determined after 1, 2 to 3, and 6 to 7 days' exposure. Similar procedures were used in conducting aging tests with the more promising insecticides. Usually these tests were conducted biweekly during the first 2 months and monthly thereafter, until live larvae were observed after the 6- to 7-day exposure. All tests were conducted in quadruplicate at room temperatures with 10 insects per replication.

Results and Discussion.—In this study a moribund larva is not classified as a living larva, and the chemical is classified as effective as long as no live larvae are observed after the 6- to 7-day continuous exposure.

During the initial exposure, live larvae of *T. inclusum* were observed after the 6- to 7-day exposure to all chemicals evaluated (table 1), with the exception of lindane in combination with Arochlor 5460, malathion alone and in combination with piperonyl butoxide, Mercaphos (3% of malathion in a neutral water emulsion), Diazinon, and Chlorthion. Over 60% of the larvae exposed to lindane, Dow ET-15, chlordane, Bayer 17450 (phosphorothioic acid, *O,O*-diethyl *O*-phthalimido ester) and aldrin at 50 mg./sq.ft. and pyrethrins at 10 in combination with piperonyl butoxide at 100 mg./sq.ft. were also moribund or dead. Dipterex, allethrin in combination with piperonyl butoxide, Bayer 18935 (phosphorothioic acid, *O,O*-diethyl *O*-2-(diethylaminoethyl) ester) and Bayer 19596 (phosphorodithioic acid, 5-(ethoxycarbonylcarbamoyl) methyl *O,O*-diethyl ester) were the least effective.

All black carpet beetle larvae exposed for 6 to 7 days to lindane, Diazinon, and malathion alone or in combination

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[2] This laboratory is a field station of the Stored-Product Insects Section, Biological Sciences Branch, Marketing Research Division, Agricultural Marketing Service, U. S. Department of Agriculture. J. E. Gorman, of this laboratory, assisted in these tests.

Table 1.—Percentage of moribund and dead larvae of *Trogoderma inclusum* after a continuous exposure to masonite panels beginning 1 hour after treatment with various insecticides.

Insecticide and Dosage (mg./sq. ft.)	Days Exposed					
	1		2 to 3		6 to 7	
	Moribund	Dead	Moribund	Dead	Moribund	Dead
Acetone	0	0	5	5	5	10
Aldrin						
50	35	2.5	65	0	27.5	37.5
200	40	2.5	62	5	37.5	27.5
Allethrin+piperonyl butoxide						
5+ 50	0	0	7.5	2.5	2.5	7.5
10+100	0	0	5	2.5	5	5
Arochlor 5460						
50	0	0	0	0	0	2.5
200	0	0	0	0	0	0
Bayer 18935						
50	0	0	10.2	5.1	10.3	5.1
200	2.5	0	17.9	7.7	2.6	7.9
Bayer 19596						
50	5	0	10	5	7.9	7.9
200	5	0	22.2	8.3	5.6	11.1

(*Continued next page*)

Table 1.—(*Continued*)

Insecticide and Dosage (mg./sq. ft.)	Days Exposed: 1 Moribund	1 Dead	2 to 3 Moribund	2 to 3 Dead	6 to 7 Moribund	6 to 7 Dead
Bayer 17450						
50	15.8	7.9	11.1	36.1	11.1	58.3
200	17.5	7.5	15	30	10	62.5
Chlordane						
50	0	0	45	5	45	22.5
200	12.5	0	50	7.5	37.5	27.5
Chlorthion						
50	32.5	0	45	35	17.5	82.5
200	30	2.5	67.5	32.5	22.5	77.5
DDT						
50	0	0	20	7.5	22.5	10
200	5	0	25	10	37.5	15
Diazinon						
50	80	20	62.5	37.5	5	95
200	62.5	37.5	50	50	0	100
Dieldrin						
50	20	0	60	7.5	22.5	35
200	10	0	67.5	7.5	35.9	25.6
Dipterex						
50	0	0	0	7.5	2.5	5
200	0	0	5	7.5	0	0
Dow ET-57 (Korlan)						
50	10	0	—	—	12.5	0
200	20	2.5	—	—	17.5	37.5
Dow ET-15						
50	2.5	0	—	—	25	37.5
200	7.5	2.5	—	—	35	42.5
Hercules AC 426-A[a]						
50	0	0	2.5	0	32.5	5
200	15	0	17.5	2.5	57.5	25
Lindane						
25	7.5	0	30	2.5	42.5	12.5
50	80	0	70	0	70	22.5
200	75	2.5	65	7.5	45	50
Lindane+Arochlor 5460						
50+ 50	72.5	5	72.5	7.5	32.5	67.5
200+200	72.5	0	80	2.5	27.5	72.5
Lindane+methoxychlor						
25+25	22.5	0	25	5	40	20
Malathion						
50	42.5	0	30	25	30	70
100	50	0	62.5	5	30	70
200	25	0	52.5	22.5	45	55
Malathion+piperonyl butoxide						
100+10	42.5	2.5	62.5	2.5	52.5	47.5
100+50	45	0	70	0	40	60
Mercaphos						
50	25	2.5	37.5	25	32.5	67.5
200	25	0	55	27.5	35	65
Methoxychlor						
25	0	0	0	0	7.5	0
50	0	2.5	12.5	5	12.5	17.5
200	5	2.5	5	2.5	10	17.5
Phostex						
50	0	0	5	5	5	12.5
200	12.5	2.5	17.5	5	5	27.5
Perthane						
50	12.5	0	10	0	5	5
200	15	2.5	3.3	0	17.5	12.5
Pyrethrins+piperonyl butoxide						
5+ 50	12.5	15	12.5	0	22.5	12.5
10+100	60	20	30	0	42.5	50
Strobane						
50	2.5	0	0	0	13.3	6.7
200	2.5	0	2.5	0	20	15
Vancide F-1562[b]						
50	0	0	0	0	2.5	15
200	0	0	0	0	25	22.5

[a] Bicyclo [2.2.1] hept-2-ene, 5-(dichloromethyl)-1,2,3,4,7,7-hexachloro-).
[b] N-Butyl nitroso methyl carbamate.

Table 2.—Percentage of moribund and dead larvae of the black carpet beetle after a continuous exposure to masonite panels beginning 1 hour after treatment with various insecticides.

Insecticide and Dosage (mg./sq. ft.)	Days Exposed: 1 Moribund	1 Dead	2 to 3 Moribund	2 to 3 Dead	6 to 7 Moribund	6 to 7 Dead
Acetone	0	0	0	0	0	0
Diazinon						
50	87.5	0	97.5	2.5	30	70
200	90	0	92.5	7.5	35	65
Lindane						
50	60	0	75	5	32.5	67.5
200	80	0	90	5	17.5	82.5
Malathion						
50	82.5	2.5	92.5	7.5	20	80
100	82.5	0	85	7.5	27.5	72.5
200	90	5	87.5	12.5	12.5	87.5
Malathion+piperonyl butoxide						
100+10	90	2.5	80	12.5	27.5	72.5
100+50	97.5	0	82.5	15	35	65

with piperonyl butoxide, were also moribund or dead during the initial test (table 2).

Malathion retained its residual toxicity to both species for the maximum period of time. Alone at 100 mg./sq.ft.

Table 3.—Percentage of moribund and dead larvae of *Trogoderma inclusum* after a 6- to 7-day exposure to masonite panels beginning 1 hour after treatment with various insecticides.

Insecticide and Dosage (mg./sq. ft.)	Age of Panels (Weeks): 2	4	5 to 6	7 to 8	12 to 13	21 to 22
Acetone	0	10	0	0	5	21.3
Aldrin						
50	37.5	—	—	—	—	—
200	90	—	—	—	—	—
Arochlor 5460						
50	0	—	—	—	—	—
200	0	—	—	—	—	—
Chlorthion						
50	100	100	80	82.5	77.5	57.5
200	100	100	100	100	100	97.5
Diazinon						
50	100	100	75	60	35	—
200	100	100	100	77.5	80	—
Dieldrin						
50	95	—	—	—	—	—
200	90	—	—	—	—	—
Lindane						
50	71.8	60	—	—	—	—
200	97.5	100	97.5	87.5	12.5	—
Lindane+Arochlor 5460						
50+ 50	52.5	—	—	—	—	—
200+200	65	—	—	—	—	—
Malathion						
50	100	87.5	100	100	97.5	—
100	100	100	100	100	100	84.6
200	100	100	100	100	95	92.5
Malathion+piperonyl butoxide						
100+10	100	100	100	100	100	82.5
100+50	100	100	100	100	100	95
Mercaphos						
50	100	85	100	100	87.5	—
200	100	100	100	100	90	—

it appeared effective against *T. inclusum* for at least 3 months and against the black carpet beetle for 5 months (tables 3 and 4). The addition of piperonyl butoxide increased the length of the residual toxicity of malathion. Diazinon at 50 mg./sq.ft. appeared to be effective against both species and Chlorthion at a similar concentration against *T. inclusum* for approximately 1 month. Live insects were noted after exposure to any panels aged after treatment with lindane at 50 mg./sq.ft. The addition of Arochlor 5460 did not increase the residual toxicity of this insecticide. Aldrin and dieldrin also were not effective after aging. Malathion, Chlorthion, Diazinon, and lindane, in the order named, therefore, appear to be the most effective insecticides evaluated to date for the control of the various species of dermestids which might occur in dry milk plants and warehouses. Lindane, Diazinon, and malathion are currently being tested for their effectiveness under natural warehouse and plant conditions.

Table 4.—Percentage of moribund and dead larvae of black carpet beetle after a 6- to 7-day exposure to masonite panels beginning 1 hour after treatment with various insecticides.

Insecticide and Dosage (mg./sq. ft.)	Age of Panels (weeks)					
	2	4	5 to 6	7 to 8	13 to 14	20 to 21
Acetone	7.5	2.5	0	0	0	0
Diazinon						
50	100	100	95	45	45	—
200	100	100	100	100	100	87.5
Lindane						
50	65	85	67.5	17.5	0	—
200	100	100	100	90	62.5	—
Malathion						
50	100	100	100	100	100	97.5
100	100	100	100	100	100	100
200	100	100	100	100	100	100
Malathion+piperonyl butoxide						
100+10	100	100	100	100	100	100
100+50	100	100	100	100	100	100

A Mating Study of the Female House Fly[1]

Louise D. Zingrone, Willis N. Bruce and George C. Decker[2]

Preliminary studies using resistant and non-resistant house flies were initiated in 1954 (Lichtwardt *et al.* 1955). A more extensive study of the mating habits of the female house fly, *Musca domestica* L., has been delayed by the absence of reliable distinctive markers. However, a green eye color was found in a field strain by this laboratory and was isolated into a pure strain. This strain was the same as that obtained from us by Ward & Hammen (1957). In our study, the green eye color was found to be a simple autosomal recessive.

The object of this study was to determine whether the female house fly would mate more than once and obtain answers for two additional questions: (1) If the female does mate more than once, at what time does the second mating occur; and (2) for what length of time does the first fertilization last?

The red-brown eye color, which is dominant and generally considered normal, and the green eye color were used for this work. The technique and general breeding methods employed were those described by Lichtwardt *et al.* (1955). The three procedures used were as follows.

Procedures.—1. In the first experiment, virgin normal-eyed females were mated with normal-eyed males in single pairs. After the normal-eyed female had been fertilized by the normal-eyed male (shown by the hatching of the eggs laid), the normal-eyed male was removed and replaced by a green-eyed male. Eggs were taken every day from this second mating for the life of the female and inoculated in medium. The F_1 from each female for each day was then mated ($F_1 \times F_1$) for the F_2. If the F_2 showed green eyes, indication was that the normal-eyed female had mated for a second time with the green-eyed male.

This procedure was used one time with 15 females. All these females produced progeny. No green eyes were found in the F_2.

2. In the second test, virgin green-eyed females were mated with green-eyed males in single pairs. When fertilization was noted, the green-eyed male was replaced by a normal-eyed male. Eggs were taken from this second mating as in the first test. If the F_1 showed normal eyes, it would indicate that the green-eyed female had mated for a second time with the normal-eyed male.

This procedure was used five times with a total of 65 females. Of this total, 56 produced progeny. The progeny of two females showed normal eyes, indicating that a second mating had taken place.

3. In the third test, virgin green-eyed females were mated with normal-eyed males in single pairs. When fertilization was noted as mentioned above, the normal-eyed male was replaced by a green-eyed male. Eggs were taken from this second mating as in the first experiment. If the F_1 showed green eyes, indication was that the green-eyed female had mated for a second time with the green-eyed male.

This procedure was used four times with a total of 50 females. Of this total, 29 produced progeny. No green eyes were found in the F_1.

Results.—A total of 130 females was used in the three experiments. Of this total, 100 produced progeny, and of the progeny only those from two females, or 2%, showed evidence of mating for the second time. Both of these second matings occurred with a green-eyed female that had first been mated with a green-eyed male and then a normal-eyed male.

When the second mating did occur, it was shortly after the second male was put in with the female. Evidence of this was found in the first egg batch taken from the second mating.

The first fertilization lasts for the entire egg-laying period of the female. This was evident in all the females, and those that mated twice produced both green-eyed and normal-eyed progeny each day.

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Laboratory Evaluations of Various Residual Sprays for the Control of Cheese Mites[1]

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Abstract

A number of chemicals were evaluated in the laboratory to determine their effectiveness at various temperatures in the control of several species of mites, the major pests of cheese held in storage for prolonged periods of time. Lindane, Diazinon, and synergized pyrethrins appear to be the most promising acaricides tested to date for the prevention of cheese contamination and damage. In general, most of the chemicals tested appeared to be the least effective at the lower temperatures. However, the low temperatures tended to delay somewhat the decrease in the effectiveness of the residual toxicity due to aging. The several species of mites used in this study exhibited varying resistance to acaricides.

Further studies on the possibility of harmful residues or off-flavors occurring if acaricides are used in close proximity to stored cheese must be conducted before final recommendations can be made.

The major pests of stored cheese are undoubtedly the various species of mites frequently found wherever cheese is held for any length of time. Since a number of mites can exist on a variety of hosts and are so widespread in nature, it appears that most cheese warehouses are confronted with the mite problem at some time. Moreover, increased cheese production in recent years and the storage of large surpluses have accentuated the need for additional research on storage problems. The infestation of cheese by mites may result in condemnation of the product by the Food and Drug Administration, unfavorable publicity for the dairy industry, and considerable damage to the cheese itself.

Mites are easily disseminated and readily escape detection until damage becomes apparent, at which time a large population has usually developed. Since industry is in urgent need of an effective spray to aid in the prevention of such dissemination and infestation, a number of chemicals have been evaluated for use as residual acaricides. However, the possible effect of any residual spray on the quality of the stored cheese and the potential presence of any harmful residues have yet to be determined for any of the chemicals evaluated in this study.

Although a number of species of mites are known to infest cheese, all tests were conducted with *Acarus siro* and *Tyrophagus* spp., which were usually the species most frequently found in the cheese warehouses visited.

Materials and Methods.—Masonite panels, 6″×6″, were treated by means of a pipette with an acetone solution of the chemical to be evaluated. With the exception of pyrethrins, allethrin, and cyclethrin, all acaricides were applied at the rate of 50 and 200 mg./sq.ft. Lindane and Diazinon were also evaluated at 10 and 25 mg./sq.ft. and malathion at 100 mg./sq.ft. Pyrethrins, allethrin, and cyclethrin were tested only at 5 and 10 mg./sq.ft. in combination with piperonyl butoxide at 50 and 100 mg./sq.ft., respectively. The latter was also evaluated as a synergist for malathion, and Arochlor 5460 (a chlorinated biphenyl) as an extender for lindane and Diazinon.

Approximately 150 test mites were exposed to panels 1 hour after treatment and were confined to the treated surface for 6 or 7 days by means of Tanglefoot. The percentage of moribund and dead mites was usually determined after 1 and 2, 3 or 4, and 6 to 7 days' exposure. Results after a 3- or 4-day exposure are not too significant as a large number of mites are trapped in the Tanglefoot. In the accompanying tables only results of the 2- to 3- or 3- to 4-day exposure are usually given. Similar procedures were used in conducting aging tests with the more promis-

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[2] This is one of the field stations of the Stored-Product Insects Section, Biological Sciences Branch, Marketing Research Division, Agricultural Marketing Service, U. S. Department of Agriculture. J. E. Gorman, of this laboratory, assisted in these tests.

Table 1.—Percentage of moribund and dead *Acarus siro* after a 3-day exposure at 35° F. on masonite panels treated with various acaricides.

Acaricide and Dosage (mg./sq. ft.)	Age of Panels in Weeks					
	0	1	3	5 to 5	7 to 8	9 to 10
Acetone	0[a]	0	0	16.8	18.3	15.2
Allethrin+piperonyl butoxide						
5+ 50	7.1	49.1	50.9	64.9	60	—
10+100	5.4	33.4	42.9	53.6	86.5	—
Arochlor 5460						
50	7.9	0	7.7	—	—	—
200	8.7	34.4	15.8	—	—	—
Cyclethrin+piperonyl butoxide						
5+ 50	56.3	33.7	—	—	—	—
10+100	45.8	46	—	—	—	—
Diazinon						
50	87.2	96.3	98.3	82.3	66.4	—
200	80.2	88.7	99.7	94.7	98	92.9
Diazinon+Arochlor 5460						
50+ 50	94.9	81.9	94.5	84.9	70.2	—
200+200	91.2	100	99	100	95.2	96
Dow ET-57 (Korlan)						
50	4.4[a]	—	—	—	—	—
200	6.5[a]	—	—	—	—	—
Dow ET-15						
50	0[a]	—	—	—	—	—
200	0[a]	—	—	—	—	—
Hercules AC 426-A						
50	56.5[a]	46.9	36.6	31	33.4	—
200	79.4[a]	95.2	90.3	82.8	81.1	70.7
Lindane						
50	100	100	100	99.6	94.7	75.7
200	100	100	100	100	100	100
Lindane+Arochlor 5460						
50 +50	—	100	99.6	99.7	100	97.6
200+200	—	100	100	100	100	100
Pyrethrins+piperonyl butoxide						
5+ 50	24.2	43.6	42.9	54.8	74	75.4
10+100	18.2	32.9	64.5	63.7	77.3	70

[a] 2-day exposure.

Table 2.—Percentage of moribund and dead *Acarus siro* after a 3- to 4-day exposure at 45° F. on masonite panels treated with various acaricides.

Acaricide and Dosage (mg./sq. ft.)	Age of Panels in Weeks					
	0	1	2	4	7	11
Acetone	5.4	2.6	7.2	9.9	2.4	7.9
Aldrin						
50	100	100	95.9	99.1	47.4	—
200	100	100	100	100	41.1	—
Allethrin+piperonyl butoxide						
5+ 50	78.9	66.2	—	—	—	—
10+100	100	94.3	—	—	—	—
Bayer 17450						
50	18.5	—	—	—	—	—
200	18.2	—	—	—	—	—
Bayer 18935						
50	38	—	—	—	—	—
200	38.5	—	—	—	—	—
Bayer 19596						
50	28	—	—	—	—	—
200	18.4	—	—	—	—	—
Chlordane						
50	65	46.7	—	—	—	—
200	58.4	34.8	—	—	—	—
Cyclethrin+piperonyl butoxide						
5+ 50	76	53.4	—	—	—	—
10+100	100	76.9	—	—	—	—
DDT						
50	86.2	81.3	73	—	—	—
200	85.1	57.9	65	—	—	—
Diazinon						
50	100	100	89.9	31.4	—	—
200	100	100	92.2	100	100	—
Dieldrin						
50	100	100	100	100	88.2	—
200	100	100	100	100	100	95.7
Dow ET-57						
50	100	73.3	—	—	—	—
200	100	85.7	—	—	—	—
Dow ET-15						
50	50.6	41.7	32.5	—	—	—
200	100	38.4	51.3	—	—	—
Hercules AC 426-A						
50	100	100	79.4	25.2	—	—
200	100	100	100	96.9	41.3	—
Lindane						
50	100	100	100	100	24	—
200	100	100	100	100	91.8	95.4
Malathion						
50	24.2	—	—	—	—	—
200	13.9	—	—	—	—	—
Methoxychlor						
50	39.5	—	—	—	—	—
200	42.5	—	—	—	—	—
Phostex						
50	20.8	—	—	—	—	—
200	35.5	—	—	—	—	—
Pyrethrins+piperonyl butoxide						
5+ 50	100	52.9	—	—	—	—
10+100	100	74.4	—	—	—	—
Sorbic acid						
50	11	—	—	—	—	—
200	9.9	—	—	—	—	—
Strobane						
50	78.8	100	83.9	91.4	—	—
200	100	100	100	100	94.2	—

Table 3.—Percentage of moribund and dead *Acarus siro* after a 3- to 4-day exposure at 55° F. on masonite panels treated with various acaricides.

Acaricide and Dosage (mg./sq. ft.)	Age of Panels in Weeks			
	0	2	4 to 5	7 to 8
Acetone	0	0	0	0
Allethrin+piperonyl butoxide				
5+ 50	35.2	—	—	—
10+100	33.5	—	—	—
Chlorthion				
50	0	—	—	—
200	0	—	—	—
Cyclethrin+piperonyl butoxide				
10+100	58.5	—	—	—
Diazinon				
10	99.1[a]	16.6[b]	—	—
25	100[a]	43.5[b]	—	—
50	100	100	—	51.6
200	100	—	—	—
Dipterex				
50	0.5	—	—	—
200	14.8[a]	—	—	—
Dow ET-57				
50	14	—	—	—
200	100	82	22.9	41
Dow ET-15				
50	0	—	—	—
200	3.2	—	—	—
Hercules AC 426-A				
200	99.7	81.8	59.1	61
Lindane				
10	99.2[a]	48.3[b]	—	2
25	100[a]	83	54.1	28.7
50	100	100	99	8.1
200	100	100	100	99.3
Malathion				
50	20	0	—	—
100	8.4[a]	—	—	—
200	8.8[a]	—	—	—
Malathion+piperonyl butoxide				
100+10	9.2[a]	—	—	—
100+50	7[a]	—	—	—
Perthane				
50	12.8[a]	—	—	—
200	78.5	—	—	—
Piperonyl butoxide				
100	.5	—	—	—
Pyrethrins				
10	18.5	6.4	—	—
Pyrethrins+piperonyl butoxide				
5+ 50	63.2	62.2	51.9	—
10+100	63.8	65.5	65.9	—
Sodium chloride				
200	0	—	—	—
Sorbic acid				
50	9.8[a]	—	—	—
200	0[a]	—	—	—
Strobane	100	99.7	44.7	—
200	100	97.9	90.4	96.5
Vancide F-1562				
50	0	—	—	—
200	5	—	—	—

[a] 2 days' exposure.
[b] 1 week's aging.

ing acaricides. All tests were conducted in quadruplicate at temperatures of 35°, 45°, and 55° F.

RESULTS.—In this study a moribund mite was not classified as a living mite and a chemical was classified as effective as long as no live mites were observed after a 3- or 4-day exposure to the treated panels. Only those chemicals which appeared effective during their initial evaluation at one of the temperatures were revaluated at the others.

Lindane was the most effective of the acaricides tested at all temperatures (tables 1, 2, and 3). At 35° F. this was followed in order by Diazinon and Hercules AC 426-A (Bicyclo [2,2,1] hept-2-ene, 5-(dichloromethyl)-1,2,3,4,7,7-hexachloro-); at 45° by Diazinon, Hercules AC 426-A,

Table 4.—Percentage of dead and moribund *Acarus siro* after a 2- to 3-day exposure at 55°, 45°, and 35° F. on masonite panels treated with various acaricides.

Acaricide and Dosage (mg./sq. ft.)	Temperature (° F.)		
	55	45	35
Acetone	0	0	0
Allethrin+piperonyl butoxide 10+100	27.5	41.2	14.5
Cyclethrin+piperonyl butoxide 10+100	—	53.7	43.1
Diazinon 50	100	100	87.2
Dow ET-57 50	0	48.4	4.4
Dow ET-15 50	0	38.4	0
Hercules AC 426-A 50	—	100	56.5
Lindane 50	100	100	100
Pyrethrins+piperonyl butoxide 10+100	45	62.4	13.6
Strobane 50	96.5	77.1	—

dieldrin, aldrin, and pyrethrins in combination with piperonyl butoxide; and at 55° by Diazinon and Strobane. Malathion, methoxychlor, sorbic acid, Phostex, Bayer 18935 (phosphorothioic acid, *O,O*-diethyl *O*-phthalimide ester), Bayer 19596 (phosphorothioic acid, *S*-(ethoxycarbonylcarbamoyl) methyl *O,O*-diethyl ester), Bayer 17450 (phosphorothioic acid, *O,O*-diethyl *O*, phthalimido ester), Chlorthion, Vancide F-1562 (N-Butyl nitroso methyl carbamate), and Dipterex were the least effective of the chemicals.

In one of the first series of tests Dow ET-57 (Korlan) and Dow ET-15 appeared effective at the higher temperatures but exhibited no significant toxicity at 35° F. In later tests these chemicals were also not significantly toxic even at the higher temperatures.

In general, most of the acaricides appeared to be the least effective at 35° F. and the most effective at 45° (table 4). The low toxicity at the former may be attributable to the minimal mite activity observed at this temperature. At 45° mite activity has significantly increased but does not reach an optimum until the higher temperatures for cheese storage. At 55°, the more favorable environment for the mites may, therefore, counteract somewhat the effect of the increased temperature on the acaricide.

Table 6.—Percentage of dead and moribund *Acarus siro* and *Tyrophagus* spp. after various periods of exposure at 55° F. on masonite panels treated with various concentrations of acaricides.

Acaricide and Dosage (mg./sq. ft.)	Length of Exposure in Days					
	1		2 to 3		6 to 7	
	Acarus siro	*Tyrophagus* spp.	*Acarus siro*	*Tyrophagus* spp.	*Acarus siro*	*Tyrophagus* spp.
Acetone	1	8.1	1.8	9.1	3	10.4
Allethrin+piperonyl butoxide						
5+ 50	7.1	21.3	35.2	37.2	50.2	38.1
10+100	9.8	12.4	33.5	21.9	64.1	32.6
Diazinon, 25	94.4	—	100	64.4	100	83.2
Dow ET-57						
50	0	.8	—	—	57.2	93.1
200	—	—	85.8	97.7	100	100
Hercules AC 426-A						
50	—	25.4	—	—	—	96.8
200	—	—	99.7	100	100	100
Lindane						
25	99	96.2	100	80	100	71
200	100	—	100	100	100	100
Malathion, 50	0	0	4.8	—	99.4	75.4
Pyrethrins+piperonyl butoxide						
5+ 50	32.8	18.1	63.2	41.8	84.4	37.6
10+100	19.7	11.4	63.8	28.5	94.3	40.8
Strobane						
50	92.5	22.1	96.5	—	100	94.6
200	—	—	100	96.1	100	99.3

At 35° F., lindane at 50 mg./sq.ft. was effective for approximately 3 weeks; at 45°, 4 weeks; and at 55°, between 2 and 4 weeks. At 25 mg./sq.ft., it was not effective after aging. Diazinon at 50 mg./sq.ft. was effective for 1 to 2 weeks at the higher temperatures. The addition of Arochlor 5460 increased the residual toxicity of these chemicals only slightly. At 45° F., aldrin and dieldrin at 50 mg./sq.ft. were effective 1 and 4 weeks, respectively, but due to their high mammalian toxicity were not investigated further. Live mites were observed after the 3- to 4-day exposure to any panels aged after treatment with synergized pyrethrins.

Lower temperatures tend to delay somewhat the decrease in effectiveness of the more toxic acaricides due to aging (table 5).

Acarus siro, the most common mite attacking cheese,

Table 5.—Percentage of dead and moribund *Acarus siro* after a 2- to 3-day exposure at 55°, 45°, and 35° F. on masonite panels which had aged for various periods of time after treatment with a number of acaricides.

Acaricide and Dosage (mg./sq. ft.)	Age of Panels in Weeks and Temperature (° F.)											
	1			2 to 3			4			7		
	55	45	35	55	45	35	55	45	35	55	45	35
Acetone	0	5.6	0	0	17.7	1.9	0	3.3	16.4	0	25.4	17.2
Allethrin+piperonyl butoxide, 10+100	—	94.8	17.2	—	2.9	36.4	—	95.2	56.6	—	—	—
Diazinon, 50	—	100	96.3	—	100	98.3	—	—	—	51.6	—	66.4
Hercules AC 426-A, 50	—	100	46.9	—	81.4	47.6	—	25.2	50.6	—	—	—
Lindane, 50	100	100	100	100	98.7	100	100	100	100	8.1	24	84.7
Pyrethrins+piperonyl butoxide, 10+100	50.8	36.9	27.2	38.5	4.8	42.5	65.9	100	86.9	—	—	—
Strobane, 50	—	93	100	100	83.9	—	—	—	—	—	—	—

Table 7.—Percentage of dead and moribund *Acarus siro* and *Tyrophagus* spp. after a 2- to 3-day exposure at 55° F. on masonite panels aged for various periods of time after treatment with a number of acaricides at 200 mg./sq. ft.

Acaricide	Age of Panels in Weeks			
	1		5	
	Acarus siro	*Tyrophagus* spp.	*Acarus siro*	*Tyrophagus* spp.
Acetone	6.8	7.9	11.7	6.6
Dow ET-57	89.1	95.3	22.9	19.4
Hercules AC 426-A	92.6	96.9	59.1	26.1
Lindane	100	100	100	83.5
Strobane	97	97.5	90.4	51.1

appeared to be less resistant to acaricides than *Tyrophagus* spp. (table 6). This was especially noted in the case of synergized pyrethrins. After the panels treated with the acaricides listed in table 7 had aged 5 weeks, all were more toxic to *A. siro* than to *Tyrophagus* spp

DISCUSSION.—Lindane, Diazinon, and synergized pyrethrins appear to date to be the most promising chemicals for further investigations in the practical application of sprays in the prevention of mite contamination of cheese. Although lindane and Diazinon were more effective as acaricides than pyrethrins, the lower mammalian toxicity of the latter presages a more verstaile use in areas which may be conducive to the deposit of harmful residues in stored cheese. Further studies will investigate the relationship between the hazard of residues or off-flavors occurring and the proximity of the acaricide. In addition, further information is needed on the relative effectiveness of these chemicals towards other species of mites which might be encountered on cheese. In the two species studied to date, those acaricides most effective against the one species also appeared to be the most effective against the other.

Egg Development in the Meadow Spittlebug and Hopkins' Bioclimatic Law[1]

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ABSTRACT

The occurrence of eggs within the body of the female spittle bug, *Philaenus leucophthalmus* (L.), is related to the principles expressed in the Hopkins' Bioclimatic Law. Data relative to the occurrence of eggs were gathered from 309 sets of 10 females each taken from 30 locations in Northeastern United States. The data did not conform to the expectation from Hopkins' Law. However, a prediction equation computed from the data is presented so that the number of eggs at a given time in a given location can be predicted.

During the course of investigations of the ecology of the meadow spittlebug, *Philaenus leucophthalmus* (L.), the author was presented the opportunity of testing Hopkins' Bioclimatic Law (Hopkins 1920). The phenological event used was the time of development of eggs within the female of the spittlebug. Weaver & King (1954) were unable to find differences in time of development due to geographical location in Ohio, and King (1952) was unable to induce artificially early development of eggs by exposure to low temperatures and reduced light. Since the time of those investigations the present author has been able to obtain adult female spittlebug specimens from several locations in the United States. The locations include:

Indiana:	Orleans	New York:	Minetto
	Eminence		Fulton
	Lafayette		
	Sand Farm	Oregon:	Granger
	Garrett		
Illinois:	Newton	Ohio:	Ross Co.
	Urbana		Marietta
	Morris		Pickaway Co.
	Hebron		Franklin Co.
			Coshocton
Maryland:	Beltsville		Holmesville
			Wooster
Delaware:	Newark		Van Wert
			Mahoning Co.
Pennsylvania:	New Hope		Summit Co.
			Wood Co.
Wisconsin:	Rock Co.		Geauga Co.
	Walworth Co.		Lake Co.

Several State and Federal extension and research workers cooperated in providing these specimens. Without this excellent participation this study could never have been undertaken.

The specimens were collected on a wide variety of dates from June to November. However, for the purpose of this study only those specimens collected between August 1 and September 30 were used. The Oregon collections were omitted because they are not pertinent to Hopkins' Law. The females collected in the field were preserved in Peterson's solution (KAAD), a fluid commonly used for killing and preserving insect larvae. The solution kept the adult insects in perfect condition for the gross dissection which was used to obtain the egg counts. Ten females from any location at any one time were cut open and the "mature" eggs counted. "Mature" eggs were those which had reached full size. Little difficulty was encountered in distinguishing full size eggs since the majority of the recognizable contents of the lower abdomen are mostly large eggs. The data were then recorded as eggs per 10 females on a specific date. The date

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was expressed as days after August 1. In addition to eggs and time, the latitude, longitude, and altitude of each location was recorded. The data then appeared in the form shown below.

Location	*Eggs*	*Time*	*Degrees N. Latitude*	*Degrees W. Longitude*	*Altitude in 100's Feet Above Sea Level*
Wooster, Ohio	85	47	40.75	81.92	9.20
	116	54	40.75	81.92	9.20
Eminence, Indiana	40	20	39.50	86.68	6.04
Newark, Delaware	1	17	39.67	75.75	1.12
etc.					

In all, 309 sets of 10 females each, collected between August 1 and September 30 were recorded in this manner. The 309 collections were made over a period of 4 years (1951–1954). No distinction was made for the year of collection since an equation of general prediction value was desired. After the data were recorded in the above manner a regression equation involving eggs as a dependent variable and time, latitude, longitude, and altitude as independent variables was formulated.

From certain preliminary observations and calculations there was some evidence that the time-egg relationship was not linear but exponential. This is no doubt true over the entire time of egg development, but for the period August 1 to September 30 a linear function seemed strong enough to express the relationship adequately. Equations using 1) the original data, 2) log time, and 3) log eggs and log time were computed. The equation from the original data seems most realistic and has the added advantage of being most readily interpreted.

If one assigns Y as eggs per 10 females, X_1 as time in days after August 1, X_2 as latitude minus 38, X_3 as longitude minus 74, and X_4 as altitude in hundreds of feet, the equation resulting from 309 sets of data collected turns out to be

$$\hat{Y} = -44.42 + 2.69X_1 + 13.10X_2 + 2.37X_3 - 3.47X_4$$

The standard deviations of the regression coefficients are $S_{b1} = 0.13$, $S_{b2} = 2.08$, $S_{b3} = 0.67$, $S_{b4} = 1.00$. If one applies a t test ($t_i = b_i/S_{bi}$), all of the regression coefficients are significantly different from zero.

To interpret this in the same terms as Hopkins' Bioclimatic Law one needs to have the time of a phenological event. The time of appearance of eight eggs per female has been selected as an important phenological time since this in the author's opinion is close to the time of initiation of egg deposition. Substituting 80 for Y in the above equation and solving for X_1, one obtains

$$X_1 = 46.25 - 4.87X_2 - 0.88X_3 + 1.29X_4$$

From this last equation one may now compute predicted values for the time of occurrence of the phenological event, eight eggs per female at any selected location. In addition, the regression coefficients express the number of days change in time of occurrence for a unit change in latitude, longitude, or altitude. Hopkins (1919) maintained that in the fall there would be a 4-day advance in the occurrence of a phenological event for every increase of 1 degree of latitude, decrease of 5 degrees of longitude, and increase of 400 feet of altitude. Table 1 compares these geographical effects on the predicted time of occurrence by our regression equation with that of Hopkins' Law.

Table 1.—Change required for a 4-day advance of a phenological event.

FACTOR	HOPKINS' LAW	SPITTLEBUG EGG RECORD
N. Latitude	+1°	+0.82°
W. Longitude	−5°	+4.54°
Altitude	+400 ft.	−310 ft.

While the values of the coefficients are remarkably close to Hopkins' prediction, the direction of the longitude and altitude effects are reversed. Since latitude is closely related to temperature one might conclude that egg development is initiated or fostered by cool temperatures. If such were true, the survival benefit to the insect is obvious, since the eggs, once laid, are subject to desiccation by heat and dry atmospheres. The effect of altitude because it is related to temperature would be subject to a similar interpretation, but the results are contrary to the expectation. A logical explanation of the longitude effect is not obvious.

If we use Wooster, Ohio as our geographical base and assume that eight eggs per female occur on September 7, we can compute the time of occurrence at other geographical locations using Hopkins' Law and the regression equation computed from the data. The comparison is made in table 2 for some of the locations from which the data were gathered. Hopkins' Law is not realistic for predicting the time of occurrence of egg development within the meadow spittlebug. The predicted dates from the regression equation are interesting in that they confirm the dates for proper application of fall insecticide applications in Ohio (Weaver 1952). In other areas, particularly farther north, the fall treatment method needs further field evaluation in the light of the predicted time of egg development.

Table 2.—Predicted time of occurrence of eight eggs per female by Hopkins' law and by the regression equation computed from actual data.

LOCATION	DATE PREDICTED BY	
	Hopkins' Law	Regression Equation
Orleans, Ind.	Sept. 22	Sept. 9
Newton, Ill.	23	5
Beltsville, Md.	18	9
Ross County, Ohio	17	9
Newark, Del.	14	7
Franklin Co., Ohio	13	7
Urbana, Ill.	17	2
Lafayette, Ind.	13	3
Wooster, Ohio	7	7
Sand Farm, Ind.	10	Aug. 30
Hebron, Ill.	7	22
Fulton, N. Y.	Aug. 30	21

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The Effect of Lygus and Hyaline Grass Bugs on Lettuce Seed Production[1]

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ABSTRACT

Field cage experiments with the lygus bug, *Lygus hesperus* Knight, showed that these bugs probably cause no significant injury to lettuce seed plants. Under the conditions of these experiments one to four adult female lygus bugs per plant did not result in any significant decrease in lettuce seed yield or germination over that obtained in the checks. The lygus bugs did not cause any increase in the number of abnormal lettuce seedlings, and they did not cause any reduction in the weight or size of lettuce seeds. No nymphs developed in the lygus bug cages, although female bugs were used.

It was determined that the bugs present in maturing lettuce seed fields were hyaline grass bugs, *Liorhyssus hyalinus* (Fab.). These bugs had been mistakenly called lygus bugs by seedsmen in the Sacramento Valley. McKinney's experiments from 1939 through 1942 in Arizona revealed that the hyaline grass bug is a lettuce-seed-feeding insect. He reported that it took large numbers of the hyaline grass bug per plant (400 and more) to reduce lettuce seed yields seriously, and to eliminate viable seeds.

McKinney's investigations indicated that 35 to 50 adult bugs per seed plant plus some nymphs were capable of instituting economic damage (especially to seed viability). The first lettuce seed to mature was not damaged by these bugs during an average season, but the later maturing seed was seriously damaged or destroyed.

Seedsmen in the Sacramento Valley of California were concerned about lygus bug damage to the lettuce seed crop (as well as many other vegetable seed crops). The bugs that were considered to be lygus bugs were only plentiful during the period of the later maturing lettuce seed fields in some seasons. However, there was no known report of positive lygus bug damage to the lettuce seed crop (only occasional light to moderate damage to the outer lettuce leaves of the market crop in some areas).

A detailed field cage investigation was therefore initiated to evaluate lygus bug injury to lettuce seed. During the 1956 season lygus bugs were introduced in varying numbers into a series of replicated field cages containing individual lettuce seed plants. The methods employed were essentially the same as those used to investigate lygus bug injury to the carrot seed crop (Carlson 1956). Thus, the techniques will be considered but briefly, in this paper.

It was found that the bugs that had been called lygus bugs by some of the seedsmen in the Sacramento Valley area were the hyaline grass bug, *Liorhyssus hyalinus* (Fab.).[3] These were present in an aphid control test that was run concurrently on the lettuce seed crop during the season of 1956.

LYGUS BUG DAMAGE.—*Methods.*—Lettuce seed plants were caged in the field before blooming and sprayed with a 0.015% solution of pyrethrum-rotenone for aphid control. One to four male lygus bugs, *Lygus hesperus* Knight, were introduced first on July 23, 1956 in each of nine replications per treatment. The lygus bug injury treatments investigated were: 0 lygus bugs per plant, 1 bug, 2 bugs, 3 bugs, and 4 lygus bugs per plant. After the first two bug introductions on July 23 and 25 it was obvious that even though the cages were all shaded, the males would not stay alive. Female bugs were then used until August 20, which was 28 days after the first introduction. At this time the lettuce seed plants and all seed spikes had 99% of the pappi formed; the majority of the seeds were quite mature with about one-half of the pappi fallen.

All caged plants were given a final spraying for control of aphid after the last lygus bug introduction (the checks were sprayed additionally throughout the tests). During the 28-day period of the lygus bug cage tests the number of female bugs was checked and the cages were replenished with bugs if necessary at least every other day. No pollinating insects were needed or used since the lettuce seed crop is self- and wind-pollinated.

The lettuce seed plants were cut when fully mature, a few days after the final spraying. The plants were removed to the greenhouse and allowed to dry on a table. The seed from each plant was hand-threshed and saved for later testing.

After weighing the total seed obtained from each replication, 100 seeds were counted and weighed for each treatment of every replication. The number of seeds per gram was then calculated to determine if there was any difference in the size of the seed between treatments. Germination tests were then conducted in blottered

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[3] Determined by Dr. Robert L. Usinger, Professor of Entomology, University of California, Berkeley, California.

sandwich boxes at 68° F. and 75–80% relative humidity for each treatment.

RESULTS.—It was difficult to keep even the female bugs alive in the field cages. Although all cages were shaded on the sunny side for their full length, 50 to 65% of the bugs had to be replaced at least every other day. Apparently the bugs did not feed much on the lettuce seed plants. Furthermore, no nymphs developed on these plants, so apparently no oviposition occurred.

Table 1 gives the average yields, germination, abnormal seedlings, and number of seeds per gram obtained from these investigations. There seemed to be some loss of seed yield per whole plant; this is misleading, however, since it was impossible to choose lettuce seed plants with the same number of secondary seed branches or spikes. Consequently, the number of secondary seed branches per plant was recorded and the yield of clean seed calculated per secondary seed branch.

There was no significant difference in yields between the treatments and the checks. Under the conditions of these experiments, lygus bugs did not reduce the seed yield of the lettuce seed plants.

The data also show that the germination was comparable for all treatments. The germination tests showed that germination was very high, and there were practically no empty seeds. In addition, the detailed data show no evidence of any delay in germination. After the final germination count, all seedlings were examined for abnormals (injury to the basal half of the cotyledons or the stem or both). The average number of abnormal seedlings was very low for all treatments, and was about the same for all. The data also show that there was no definite decrease in the weight or size of the seed for any of the treatments.

HYALINE GRASS BUG DAMAGE.—Observations made during the aphid control work indicated that the hyaline grass bug, *Liorhyssus hyalinus* became fairly numerous by August 15, but this species was not collected in large numbers until the last two seed plant shaggings (or shaking for seeds) of the aphid-infested lettuce seed plants. These bugs were observed to be very gregarious and appeared to be most numerous on the aphid-infested plants of the checks. They seemed to be attracted to and feeding on the honeydew excreted by the aphids. It was not possible to thoroughly check any possible feeding on the developing or maturing lettuce seeds. However, during the 1956 season these bugs did not appear to be greatly damaging to the lettuce seed crop in this area.

The biology of the hyaline grass bug was investigated by Readio (1928), who stated that the bugs seemed to prefer the reproductive parts of plants. Rearing this species on wild lettuce, he found the life cycle to be as short as 17 days, with a probability of four or five generations during a season in Pennsylvania. Readio also reported that these bugs were not confined to a single host species.

McKinney (1938) reported that preliminary cage tests indicated that a 1% rotenone-clay dust was effective in controlling adults of the hyaline grass bug in the Salt River Valley of Arizona. This treatment was reportedly more effective on adults than on nymphs, both of which were apparently causing considerable damage to lettuce seed in that area. According to McKinney (1940), satisfactory control of nymphs as well as adults was achieved with derris-sulfur-talc, but not with derris alone. It was concluded that the treatments must be applied when infestations were still low, and repeated at 4- to 5-day intervals.

Table 1.—The extent and type of lygus bug damage to lettuce seed, determined by cage tests with varying numbers of bugs per plant. Davis, California, 1956.[a]

BUGS PER PLANT	AVERAGE SEED YIELD (GM.) PER		AVERAGE RESULTS OF SEED GERMINATION		NO. OF SEEDS PER GRAM
	Plant	Secondary Seed Branch	Final Per Cent Germination	No. of Abnormal Seedlings	
0	7.89	0.806	99.9	0.44	1111.1
1	6.30	0.667	98.6	0.33	1136.3
2	6.68	0.939	98.9	0.33	1149.4
3	5.35	0.641	99.7	0.22	1162.7
4	5.80	0.829	98.8	0.44	1111.1

[a] Each of these treatments was replicated 9 times in field cages. Female bugs were used. The number of bugs per treatment was checked every other day during the blooming period (about 28 days), and additional bugs were introduced if necessary to maintain the design of the experiment.

In this article the following information on the hyaline grass bug was obtained from unpublished U. S. Department of Agriculture reports by the late K. B. McKinney. His studies were conducted on the biology and control of this insect and its damage to lettuce grown for seed at Phoenix, Arizona, from 1938 through 1942.

From the cage investigations performed by McKinney it was difficult to determine the number of bugs necessary to cause economic damage. Apparently it took over 70 adults and an unknown number of nymphs to reduce germination significantly, while the yield remained fairly satisfactory. The bug population that resulted in a severe loss of yield and very little germination was very high (400 or more adults were recovered per plant). Indications were that field-run plants produced good yields and high-germination seed early in the season (at the first seed plant shagging), some reduction in viable seed in only 6 days, and greater reduction later. McKinney stated later that an average of 35 to 50 adults per plant seemed high enough to institute economic damage to lettuce seed.

The life cycle of these bugs varied considerably, depending on the temperature. There were five nymphal instars with an occasional sixth. Eggs deposited in May (when seed was first developing and temperatures were cooler) hatched in 6 days, and nymphal development was completed in 18 to 26 days. Earlier work had shown a possible low of 4 days for eggs to hatch, and a nymphal development period of as low as 11 days at high temperatures. Nymphs that were fed on flower buds of cultivated lettuce matured in about half the time required by those fed on wild lettuce. The nymphs seemed to feed exclusively on flower buds and developing seeds. Both males and females lived longer than 2 months in breeding cages, and individual females deposited 200 or more eggs (the eggs are red and are deposited in clusters on the seed spikes). Overwintering was by adult hibernation.

The latest control work by McKinney in 1942 indi-

cated that lettuce seed plants maturing between the middle of May and the first part of July could be protected from severe hyaline grass bug damage. This was accomplished by four applications of Paris green-sulfur-lime dust at 5- to 6-day intervals, beginning May 15. At that time, bug control and germination increase were apparently just as satisfactory with sulfur alone. Later-maturing seed, after the final application of the dusts, was still seriously damaged or totally destroyed. As far as is known, present-day control is effected with DDT or toxaphene in those areas in which the bugs heavily infest the late-maturing lettuce seed crop.

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Effect of Time of Application of DDT on Lygus Bug Populations and Yield of Lima Beans[1]

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Abstract

In experiments conducted in southern California in 1952–57 a single early or blossom application and a single intermediate application in late July when small pods were present gave significant increases in yield of beans, each being superior to a single late application made in August. A late application to plots treated early did not further increase the yield. All these treatments significantly reduced the percentage of pitted beans, the intermediate and the early-plus-late applications being responsible for the greatest reductions. Peak populations of lygus during the first half of August indicated the necessity for applying an insecticide 2 weeks after blossoming, or in the latter half of July, to prevent damage to small and half-mature pods present on the plants in August. The intermediate application made about this time was the most effective in reducing corn earworm damage to pods and beans.

In southern California the damage to lima beans by lygus bugs varies from year to year, so that it is difficult for growers to decide whether control measures are practical. The principal species is *Lygus hesperus* (Kngt.), although *L. elisus* Van D. is also present. DDT has been used extensively for the control of these insects. This paper is a report of experiments conducted from 1952 through 1957, principally on the timing of DDT applications, which varied according to insect abundance and the stage of plant development.

Lima bean injury due to lygus bug attack was brought to our attention in 1944 by A. H. Holland, of the Agricultural Extension Service in Ventura County. He had observed this condition on the A. R. Noble farm near Camarillo. The plants adjacent to a field of alfalfa had grown rank and tall, had few pods, most of the beans were malformed or pitted, and large numbers of shriveled pods were on the ground. Lygus bugs were very plentiful. No pods were present on the plants within 50 feet of the alfalfa, and because of the scarcity of pods the beans within 300 feet of the alfalfa, or 10 acres, were not threshed. In the same year a similar situation prevailed on the G. Chaffee farm near Saticoy. At a distance of 50 feet from his field of alfalfa, the lima beans averaged 13 pods per plant, at 100 feet 19 pods, at 200 feet 25 pods, and at 600 feet 32 pods, which was about normal. In the next few years lygus damage in bean fields was not excessive, but in 1949 a processing plant rejected green Fordhook beans from four fields in this area because of excessive pitting. The beans were allowed to mature, but when threshed and cleaned 22% of them were classed as culls because of pitting.

Shull (1933) and Elmore (1955) have described the damage to beans by lygus bugs. An extensive review of lygus bug injury to lima beans in California is reported by Baker *et al.* (1946). Middlekauff & Stevenson (1952) reported excellent control of lygus bugs on blackeye beans.

Lygus injury occurs during most of the growing period of lima beans. On young plants ovipisition and the feeding in tender stems, fruiting spikes, and small pods may cause blossom buds or pods to drop, with consequent crop loss. On the older plants the pods are punctured and the beans are pitted and distorted and thus are unmarketable. The bugs prefer the succulent small pods 2 inches or less in length, but as the populations increase they attack green pods of all sizes. Population surveys showed that where there were more than three bugs per sweep the plants produced few pods and 9 to 22% of the beans were pitted.

The abundance of lygus bugs in lima bean fields is dependent on many conditions. The type of vegetation in the area adjacent to bean fields influences the size of lygus populations. They prefer large, green succulent plants. Uncultivated weedy areas, seed crops, and alfalfa are all favorable for their development, and they migrate from these areas to neighboring bean fields.

Methods.—Randomized-block experiments were conducted on Ventura lima beans in commercial fields in

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[2] The following growers allowed us to use their bean fields for these experiments: A. Changala, Farnsworth Bros., H. L. Daily, Clyde Casey, Willis Miller, and Griset Bros.

Table 1.—Average numbers of lygus bugs per sweep before and after applications of DDT, 1952–57.

Time of Application	1952		1953		1954		1955		1956		1957		Average	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Early	0.09	0.13	0.17	0.13	0	0.25	0.25	0.28	0.10	0.29	0.21	0.18	0.15	0.22
Intermediate	.28	.06	.23	.19	.02	.20	.19	.24	.23	.16	.27	.28	.20	.19
Late	.40	.02	.40	.02	.25	.03	.56	.07	.40	.06	.47	.05	.41	.04
Early plus late	.12	.01	.15	.07	0	.29	.20	.11	.05	.08	.14	.03	.12	.10
Untreated[a]	.30	—	.38	—	.27	—	.43	—	.42	—	.49	—	.38	—

[a] Average for season.

Orange and Los Angeles Counties. To minimize the effect of drift, the plots were large, from 24 to 36 rows wide (30 inches apart) and 150 to 200 feet long. Single applications of DDT were made at three different times—just after blossoming commenced (early), when newly formed pods appeared in late July (intermediate), and when pods were 1½ to 2 inches in length (late). On one set of plots DDT was applied both early and late.

In the experiments from 1952 through 1954 a dust containing 5% of DDT and 50% of sulfur was applied at 30 pounds per acre with a 14-row power duster between 6 and 8 A.M. In the later years 1½ pounds of DDT per acre was applied in 12 to 16 gallons of emulsion with an eight-row power sprayer operating at a pressure of 125 pounds per square inch and having a boom with two nozzles per row. To discourage the buildup of two-spotted spider mites (*Tetranychus telarius* (L.)) the entire experimental planting was sprayed at the beginning of each season with Aramite at 1 pound per acre. Aramite had been found to have no insecticidal value against lygus nymphs or adults.

The effect of the treatments on lygus populations was determined weekly by making five sweeps on the bean foliage near the center of each plot with a 15-inch insect net between 8 A.M. and 1 P.M., and counting the adults and nymphs collected. When the beans were threshed, 2-pound samples from each plot were retained and 100 to 200 beans per sample were examined for pitting or malformation. From 1955 through 1957 all pods on three to five plants per plot were picked and examined externally for injury due to the feeding of corn earworms. From 50 to 100 of these pods per plot were shelled and the beans examined for injury by lygus. Yield data were obtained by harvesting and weighing the beans from 1/100-acre areas in the center of each plot.

1952 EXPERIMENTS.—The Farnsworth field near Talbert was used for the initial tests. Each treatment was replicated six times. Lima beans had been planted on May 10. The early application of DDT was made on July 22, the intermediate application on August 5, and the late one on August 19. In one treatment both the early and late applications were included.

Lygus populations remained below one bug per sweep (table 1). Nevertheless, 2.2% of the untreated beans were pitted by lygus, and all except the late-application treatments were responsible for a significant reduction in bean pitting. There were no significant differences between treatments in yield of dry beans, but, as indiated in table 2, the treated plots had from 2.7 to 5 more sacks per acre than the check plots.

1953 EXPERIMENT.—These tests were also conducted on the Farnsworth farm, in a field planted on May 15. There were six replicates of each treatment. The early application of DDT was made on July 16, the intermediate one on July 31, and the late one on August 14. On the basis of the number of beans pitted, the single early, single intermediate, and the early-plus-late applications were superior to the single late application. Nevertheless, all dust treatments gave good control of lygus and significantly reduced the pitting. Yields in the treated plots were 0.9 to 7.4 sacks per acre more than in the untreated plots, but this difference was not significant. The highest yield was in the early-treated plots.

1954 EXPERIMENT.—These tests were conducted on the Dady farm near Greenville, in lima beans planted on May 10. Each treatment was replicated six times. DDT was applied earlier than usual—on July 2, July 20, and August 5. Lygus bugs were not very numerous in this field. Only 1.3% of the beans were pitted in the untreated plots, but all except the single late application and the

Table 2.—Effect of time of application of DDT on yield (100-pound sacks) of dry lima beans and on degree of injury, 1952–57.

Time of Application	1952		1953		1954		1955		1956		1957		Average	
	Yield	Per Cent Pitted	Yield	Per Cent Pitted	Yield	Per Cent Pitted	Yield	Per Cent Pitted	Yield	Per Cent Pitted	Yield	Per Cent Pitted	Yield	Per Cent Pitted
Early	20.8	0.87	25.4	0.25	21.3	0.50	19.0	0.29	9.9	4.75	18.2	0.95	19.1	1.07
Intermediate	19.9	1.13	20.3	.33	22.4	.08	18.0	.18	9.6	2.58	20.8	.90	18.5	.77
Late	21.6	1.53	18.9	1.08	17.2	1.00	17.7	.11	8.9	3.33	13.0	1.10	16.2	1.14
Early+late	19.1	.87	21.7	.42	20.4	.83	16.8	.21	10.0	1.67	19.3	.60	17.9	.60
Untreated	16.4	2.20	18.0	3.00	18.7	1.33	18.4	.89	10.6	6.42	17.1	1.95	16.5	2.28
L.S.D. 5%	—[a]	.88	—[a]	1.01	—[a]	.67	—[a]	.36	—[a]	2.06	4.4	.67	1.87	.39

[a] Not significant according to *F* test.

Table 3.—Numbers of lygus adults and nymphs collected at intervals in untreated plots of lima beans, 1952–57.

Dates	Adults	Nymphs	Per Cent of Seasonal Total
July 1–15	23	35	5.2
16–31	167	129	26.5
August 1–15	365	149	46.0
16–31	202	47	22.3

Table 4.—Bean pods injured by corn earworms in all fields treated with DDT, 1956 and 1957.

	1956		1957	
Time of Application	Number	Per Cent	Number	Per Cent
Early	27	4.5	170	13.6
Intermediate	1	.2	17	1.4
Late	3	.5	144	11.5
Early plus late	7	1.1	101	8.1
Untreated	27	4.5	162	13.0
L.S.D. at 5% level	14.6	2.4	77.4	6.2

early-plus-late applications significantly reduced the pitting. The yield data showed no significant difference between dust applications. The best yields were obtained from the plots treated on July 2 and July 20.

1955 Experiment.—This experiment was conducted on the Griset farm in Los Angeles County, and on the Farnsworth farm in Orange County. There were eight replicates in the Griset and six in the Farnsworth field. The Farnsworth field was planted on May 15 and the Griset field on May 10. The early application of DDT was made between July 14 and 18, the intermediate on August 2, and the late on August 18. Peak populations of more than one bug per sweep occurred on August 10 in the Griset field, and on August 17 in the Farnsworth field. In view of the similarity of the results, the data from the two fields were combined. There was an unusually small amount of pitting in both fields (1% or less), but all spray applications significantly reduced the pitting. The yield data showed no significant differences between treatments.

1956 Experiment.—These tests were conducted on the Miller farm in Los Angeles County, in a field of Mackie bush beans planted on May 20. There were 12 replicates of each treatment. DDT sprays were applied on July 11, July 30, and August 9. The early application did not give adequate control of lygus throughout the season, as populations close to 0.3 bug per sweep were responsible for 4.8% of pitted beans. The other applications significantly reduced the percentage of pitted beans. All applications reduced the average lygus population below the check, and two applications were superior to each single application. In yield of beans there were no significant differences between treatments and the check.

1957 Experiment.—These tests were conducted in the Casey field, Los Angeles County, with 10 replicates. Lima beans were planted on May 24, and the DDT sprays applied on July 4, July 19, and August 1. In this field lygus bugs were most numerous between July 15 and August 7, the peak collections being obtained on July 24 and 30. In lygus control all applications were superior to the checks. Also two applications were better than one, and the early application was better than either of the later ones. Yields from the early, intermediate, and the combination of the early and late applications were higher than those from the one late application. The highest yield was obtained with the intermediate application. All the DDT sprays reduced the extent of pitting to 1.1% of the beans or less.

Corn Earworm Injury in DDT-treated Plots.—1954 and 1955 totals of 2800 and 1200 threshed beans, respectively, were examined per treatment to determine the injury caused by the corn earworm (*Heliothis zea* (Boddie)). In 1954 this injury ranged from 0.4 to 1.4%, with no significant difference between treatments or check. In 1955 injury was less than 1%.

Earworms may consume all the beans in a pod, or leave only fragments that are lost during threshing, and this may account for the small amount of injury noted on the threshed beans. Therefore, in 1956 and 1957 the pods on five or more plants per plot were inspected for earworm injury. In 1956 all except the single early application caused significant reductions in earworm injury, and the single intermediate application gave the best control (table 4). In 1957 the intermediate application was the most effective, only 1.4% of the pods being damaged as compared with 13% in the check. With the other treatments 8 to 13.6% of the pods were injured, an indication that these DDT applications did not control this pest. Examinations of pods rather than of threshed beans showed that earworms are responsible for considerably more injury than heretofore known, and that they should be taken into account in any control program for bean insects.

Discussion.—The yield data show that single early and intermediate applications were each responsible for increases in yield of 2 to 2½ 100-pound sacks of lima beans per acre and were superior to a single late application in this respect. The late application, when pods were 1½ to 2 inches long, could be expected to be inferior because of the gradual increase and higher lygus populations. Judging by yield, the early application, which should protect blossoms from lygus injury, was not superior to the intermediate application. The late application to plots treated early did not give any further increase in yield, but did reduce pitting.

Beans from all the treated plots showed less pitting than those from the untreated check. Two applications (early plus late) and the single intermediate application were responsible for the lowest percentages of pitted beans, but the second (late) application did not reduce the damage enough to be economically justified.

In the untreated plots sweeping at weekly intervals showed the peak population (46%) between August 1 and 15. Only 5% of the seasonal population was collected during the first half of July, 26% from July 16 to 31, and 22% during the last half of August (table 3). It is therefore important to apply an insecticide during the latter half of July so as to forestall a buildup in August, when all sizes of pods are present on the plants. An application at this time also helps to reduce corn earworm damage to pods.

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Biological Notes on the Predaceous Earwig *Labidura riparia* (Pallas), a Recent Immigrant to California [Dermaptera: Labiduridae][1]

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ABSTRACT

The occurrence of a beneficial predaceous earwig in California is noted. Its rather restricted distribution in the agricultural valleys of southeastern California is given, together with reasons for this restriction. Rearing, burrow construction, mating, oviposition, longevity, feeding habits, and food preferences are discussed. Litter- or soil-inhabiting insects and lepidopterous larvae and pupae were found to be the preferred foods.

During investigations of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in the Imperial Valley, California, from 1955 to 1957, an unusually large, unrecognized earwig species was encountered in relatively great abundance within or near alfalfa fields. Specimens of this earwig were sent to Dr. A. B. Gurney, of the U. S. National Museum, Washington, D. C., who identified them as *Labidura riparia* (Pallas). This species was originally described from Siberia, and is apparently the same as *L. bidens* (Olivier), which was described from Jamaica (Gurney 1950, 1958). In reviewing the literature, one would come to the conclusion that *L. riparia* is worldwide in distribution in its restricted, tropico-subtemperate range; however, it now seems possible that at least some of its populations are subspecies (Gurney 1958, Hincks 1954).

According to Gurney (1958), *Labidura riparia* is known in the United States from Alabama, Florida, Georgia, Texas, Louisiana, and now California. He also states that specimens are frequently intercepted by plant quarantine inspectors at United States ports, and that there seem to be good possibilities for this species to establish breeding colonies in many of our warmer states. How, when, or from what place *L. riparia* came to California is unknown. However, this is the second species of earwig that has recently been observed to move into southern California, the first being the African *Euborellia cincticornis* (Gerst.), which was reported by Gurney (1950) as occurring at Blythe, Ripley, and Fort Yuma.

Labidura riparia was first collected in California at Calexico on November 21, 1952, by R. M. Hawthorne (1958), Survey Entomologist for the California Department of Agriculture. He also noted that further reports on the occurence of *L. riparia* in California were not made until 1954.

Various attempts were made by the authors during the period from 1955 to 1957 to determine the range of this species in California. At present it seems to be confined to the agricultural areas of the Bard, Imperial, and Palo Verde valleys. Specific localities where this species has been encountered include the following: Bard, Brawley, Calipatria, Calexico, Date City, El Centro, Holtville, San Felipe Creek, Seeley, Westmoreland, Winterhaven (Imperial County), and Blythe (Riverside County). It has not, however, been found in the Coachella Valley, another agricultural desert valley situated some 40 miles north of the Imperial Valley and some 80 miles west of the Palo Verde Valley. The large, dry desert expanse separating these valleys has probably served as a barrier to the northward or westward movement of this earwig.

Labidura riparia appears to be particularly abundant in alfalfa fields, especially in fields that have well-defined ridges between the planted lands. The earwigs apparently prefer these ridges to the planted areas because they are not flooded at irrigation times and yet approximate the humid microclimate created by irrigations throughout the year, a condition which seems to suit their needs. Specimens in burrows have also been observed under baled hay, under rocks, in holes along canal banks, and under eucalyptus bark close to the ground, but they have always been found in close association with rather regularly irrigated fields. *L. riparia* has at times been found in company with *Euborellia cincticornis*, the two earwigs apparently being able to live together without noticeable harm coming to either so long as food is available.

BIOLOGICAL OBSERVATIONS IN THE LABORATORY.—Although species in several genera of Labiduridae and Forficulidae have been reported as being predaceous on various insects (Clausen 1940), apparently little close observation has been made on this habit. The following notes are based on observations of colonies of *Labidura riparia* which have been maintained in the laboratory from November 1957 to April 1958 under fairly constant conditions of temperature (75° F.) and relative humidity (50%).

For one study colony of about 25 individuals of both

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[2] The assistance of the following persons is gratefully acknowledged: J. W. MacSwain, University of California, Berkeley, and R. F. Wilkey, California Department of Agriculture, for the primary determination of this earwig; A. B. Gurney, U. S. National Museum, Washington, D. C., for verification of the specific name, *Labidura riparia* (Pallas); and R. M. Hawthorne, California Department of Agriculture, for ntoes and distributional data.

sexes, a large battery jar was used which contained about 3 inches of soil that was kept continuously moist. The surface of this soil was made smooth to determine to what extent the earwigs were able to excavate their own burrows. Within a day after setting up the colony, several small holes had been dug, and although other holes were dug in the ensuing week, no large ones were noticed until a piece of cardboard was placed on the soil to give the earwigs some overhead protection. Within a week of this time, several large tunnels were excavated, and the main one, which extended down to the bottom of the jar, had several lateral branches. This main tunnel contained some widened lateral areas that served as egg-deposition sites for several different females; thus it was found that more than one individual may use a tunnel at the same time. The dirt removed from the tunnels was neatly placed outside the entrances in the form of runways, with usually more than one runway to each tunnel. These tunnels and runways were of the same construction as those found in the field, thereby indicating that *Labidura riparia* is capable of constructing rather extensive and elaborate tunnels.

Another type of hole is made when overhead protection is not available, this being a simple, single hole about as deep as the length of the earwig inhabiting it. Invariably, females found in these holes had their heads facing downward with just the tip of their caudal forceps extruding above the soil line. It was under these circumstances that mating was most often observed, being first noticed in the colony in early March 1958. In mating, the male approached the female in her hole, then quickly turned around and thrust his forceps into the burrow. Usually only after several attempts were made to contact the female in this manner was copulation effected. During copulation both sexes remained with their legs on the ground, the male's forceps usually being ventral to the female's. Mating lasted from 5 to 25 minutes. A female may mate more than once, and a second mating by another male may take place within minutes after the previous act without any sign of disinterest on the part of the female.

Eggs were laid in groups of 20 to 50, and were brooded over and moved, apparently in a fashion similar to that of certain other earwig species. Incubation required about 14 days under laboratory conditions. Our records in the laboratory indicate adult longevity of at least 5 months.

Labidura riparia apparently has at least two distinct forms in the male: one very similar to the female; the other much larger and with longer, more strongly developed forceps with pointed swellings located on the inner margins on each side about midway of their length. Observations did not permit the authors to ascertain what, if any, specific significance is related to these two forms; however, only the smaller of the two male types was observed to mate with the females.

At no time were any of the laboratory individuals observed to fly. An examination of the wings showed them to be of a flimsy and somewhat weakly formed nature, which may indicate that *Labidura riparia*, like some other species of earwigs, is unable to fly. If this is true, it may explain why *L. riparia* has not been able to cross the arid desert barriers to become more widespread in California.

FEEDING HABITS.—Apparently, there are only two records in the literature pertaining to the feeding habits of *Labidura riparia*. Bishara (1934) found that this earwig was an extensive predator of larvae of the armyworm *Prodenia litura* F. on cotton in Egypt, a single earwig being able to consume 10 to 20 larvae in one night. He also stated that *L. riparia* killed many more larvae than were actually eaten, a habit often considered characteristic of a good predator. Dean & Schuster (1958) noted in Texas that one earwig could devour 25 crawlers of Rhodesgrass scale in a 60-second period.

Our observations revealed that *Labidura riparia* feeds ravenously on nearly every type of living insect offered to it in the laboratory. The earwig seizes the larger living prey species by means of the large caudal forceps, much as a crab or scorpion seizes its prey with its chelicerae. The abdomen and forceps are quite flexible, and as the earwig approaches its motionless or slowly moving prey, a quick forward curving and thrusting of its abdomen enables the earwig to grasp the prey securely in its forceps. The earwig then rapidly attacks the freely moving part of the prey with its mandibles, and immediately begins the masticating feeding process. This is apparently quite similar to the feeding habits of *Chelisoches morio* F., as noted by Risbec (1933). Only the heavily chitinized structures of the prey remain when *L. riparia* has finished feeding. When feeding on small insects such as aphids, the mandibles and not the caudal forceps are used to seize the prey.

Insects consumed by *Labidura riparia* in the laboratory included several noctuid species (adults, pupae, and larvae), the painted-lady butterfly, *Vanessa cardui* (L.) (adults, pupae, and larvae), young mantids, elaterid larvae, larvae of two species of carabids, *Tenebrio molitor* L. (adults, pupae, and larvae), *Hypera brunneipennis* (Boh.) (pupae and larvae), *Colops* larvae, pea aphids, *Macrosiphum pisi* (Harris), and spotted alfalfa aphids, *Therioaphis maculata* (Buckton). Adult *Hippodamia* spp. apparently were able to repel the earwig through repugnatory emissions, while mantid egg cases and pupae of the salt-marsh caterpillar, *Estigmene acrea* (Drury), were apparently too tough for penetration by either the earwig's forceps or mandibles. Besides insects, earthworms were found to be a satisfactory food.

Judging from the field habits and the general attitudes shown by the earwig in the laboratory, it would seem that its preferred food consists primarily of litter- or soil-inhabiting insects, most likely the larvae and pupae of various lepidopteran species that are common in most agricultural fields. The earwigs have, however, been observed to climb alfalfa stems in the laboratory and completely consume large populations of pea aphids and spotted alfalfa aphids located thereon. Although such feeding has not been observed during the daytime in the field, it seems quite possible that the earwigs may resort to this type of feeding on warm nights.

When deprived of live prey, individuals of *Labidura riparia* have been seen to eat alfalfa seeds and bread, though with some reluctance, and death invariably followed after a prolonged diet of this type food. They were not strongly attracted to recently killed or dead insects but were observed, at times, to feed on such foods. They

occasionally fed on freshly killed bodies of their own kind, although they never completely consumed the food and at no time was cannibalism observed. Water was found to be an absolute necessity for successful rearing of these earwigs, since all individuals died in the laboratory within 10 days without available water.

Labidura riparia produces a peculiar, strong, rancid, rotting-type odor. This is most evident when an individual is first disturbed, and the odor may persist on one's hand for several hours. In the laboratory this odor persisted in the original container for about a week after the colony was established, then was no longer evident until the earwigs were greatly disturbed.

CONCLUSION.—Bishara (1934) gave some evidence that *Labidura riparia* was beneficial because of its predatory habit. The evidence presented here seems to corroborate this opinion. Apparently, *L. riparia* is one of those essentially beneficial insect species which was accidentally introduced into a new habitat and may eventually come to play an important role in the natural control of agricultural pests. Further studies will be necessary to ascertain the extent of the benefits being derived from this new immigrant.

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Influence of Feeding Time, Stylet Penetration, and Developmental Instar on the Toxic Effect of the Spotted Alfalfa Aphid[1]

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ABSTRACT

Greenhouse experiments on the production of yellow vein-banding symptoms by the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) on seedling Caliverde alfalfa plants were conducted during the spring and summer of 1958.

The percentage of plants affected with yellow vein-banding symptoms increased with an increase in the length of feeding time of individual aphids. The amount of time required for systemic symptom expression on 50% of the plants for third and fourth instar nymphs was estimated at approximately 10 hours. The symptom incidence was higher for late (third and fourth) instar than for early (first and second) instar nymphs.

Most of the variability in symptom incidence was attributed to variable plant susceptibility and not inherent differences in aphid toxicity.

T. maculata and *Myzus persicae* (Sulz.) each produced salivary sheaths when fed through a membrane into water. These sheaths were quite distinct between species in size and configuration. Experiments demonstrated that toxin injection, as measured by disease incidence, was a constant and somewhat additive function of feeding time, and was not dependent solely on the number of sheaths produced.

Attempts to isolate the toxic principle were made; however, experiments to find a satisfactory bioassay method for it were negative.

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton) causes economic losses in alfalfa by lowering the value of the hay due to excessive honeydew formation and by loss of yield and decrease in stand presumably due to (1) removal of plant juices, and (2) injection of a toxin. Dickson *et al.* (1955) discussed the types of injury and reported that small seedling alfalfa plants were sometimes killed by single aphids. Additional information on the plasmolytic effect of feeding was given by Diehl & Chatters (1956), and Paschke & Sylvester (1957) indicated that occasionally one aptera and its offspring feeding for 7 days on an alfalfa seedling (var. Africa) under greenhouse conditions could kill the plant, and they attributed this severe injury to a toxin. These workers described and illustrated the toxemia symptoms which consisted of yellow vein-banding of the leaves at or near the growing tip plus localized chlorotic lesions at the feeding sites as reported by Diehl & Chatters (1956). Paschke & Sylvester (1957) also presented evidence indicating that the disease was not due to a virus, but rather met the definition requirements of an insect-induced plant toxemia. The observations reported herein were made in an attempt to learn more of the nature of the toxin injection and subsequent plant response.

MATERIALS AND METHODS.—Experiments were conducted during the spring and summer of 1958 under greenhouse conditions. Test plants were alfalfa seedlings (*Medicago sativa* L. var. Caliverde, foundation seed) which had been sprouted in sterile sand and then trans-

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planted to 3-inch clay pots. In most cases the test plants were used when the first trifoliate leaf was partially to fully expanded. Test aphids were taken from a stock colony maintained on potted alfalfa plants in the greenhouse and which had been initiated several years ago from field collections. Single insects were transferred with a micropipette.

Readings for symptoms were qualitative and plants were considered positive or negative on the basis of whether they displayed detectable yellow vein-banding symptoms on two or more of the disease reading occasions, except in the clonal toxicity experiment in which plants displaying symptoms in one of the two readings were counted as positive.

RESULTS.—*Preliminary.*—Repeated observations on symptom development confirmed for variety Caliverde the findings of Paschke & Sylvester (1957) for variety Africa. Regardless of where on the plants the aphids were allowed to feed the vein-banding symptoms appeared only in new growth, usually in the next trifoliate leaf to expand. First symptoms usually became evident after about 48 hours from the time of infestation, although occasionally they could be seen as early as 24 hours. After removal of the aphids, the symptoms persisted for several days in the affected leaves, although they became progressively weaker in intensity. The subsequently developing leaves were usually free of symptoms.

Feeding Time and Symptom Incidence.—Unpublished results of Paschke & Sylvester suggested that local spotting symptoms could result from a minimum of 5 minutes, and vein-banding symptoms from a minimum of 30 minutes, of feeding by single apterae. The results also indicated that the percentage of exposed plants displaying symptoms varied with the period of infestation.

To test for the relation of exposure time to the incidence of plants displaying yellow vein-banding symptoms, single, third or fourth instar spotted alfalfa aphid nymphs were placed on alfalfa seedlings and then removed after periods of 23, 100, and 1000 minutes. A check group of plants was kept free of aphids. Ten plants were used for each treatment and the test was replicated four times. Disease readings were made on the second, third, and fourth days following exposure, noting whether definite yellow vein-banding symptoms were present or absent. No disease occurred in the uninfested check plants.

The results are given in table 1. Assuming linearity a regression line was calculated (fig. 1) and from this the 50% level of disease incidence was estimated to occur with approximately 10 hours of feeding by single insects. However, related observations have indicated that the 50% level will vary considerably with age and growing conditions of the plants, and possibly also with temperature, and thus the procedure has but limited usefulness in establishing a dosage standard for assessment purposes.

Table 1.—Results of trials to determine the influence of various access times of single late instar *Therioaphis maculata* nymphs to Caliverde alfalfa seedlings on the incidence of systemic toxic symptoms.

ACCESS TIME (MIN.)	NUMBER OF PLANTS		PER CENT POSITIVE
	Tested	Positive[a]	
0[b]	40[c]	0	0.0
23	40	2	5.0
100	40	4	10.0
1000	40	31	77.5

[a] A minimum of two out of three possible positive readings made on successive days was required before the plant was recorded as positive.
[b] Check series, no insects placed on plants.
[c] Ten plants per treatment, four replications.

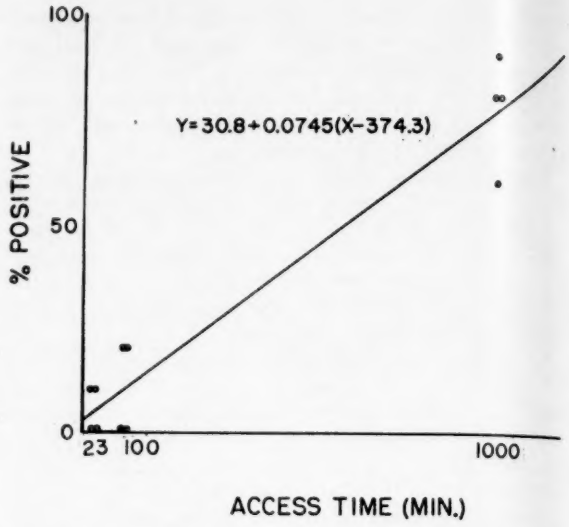

FIG. 1.—Influence of various access times of single, late instar *Therioaphis maculata* nymphs to Caliverde alfalfa seedlings on the incidence of systemic toxic symptoms.

It is not known how the qualitative recordings are related to the quantitative aspects of the toxin, nor even if they indicate presence or absence of it. They perhaps only indicate the presence of the toxin in amounts needed to exceed the threshold for symptom expression under certain specified conditions. In spite of the obvious limitations, this method of evaluation provided a somewhat useful comparative tool in further tests regarding the relative toxicity of different instars and aphid clones.

Comparative Toxicity of Nymphal Instars.—A preliminary experiment indicated that nymphs in later instars (third and fourth) were more toxic than those in early instars (first and second). There also were indications in these exploratory tests that the first instar nymphs were more toxic than second instar nymphs. To test these trends further, an experiment was designed in which first, second, and pooled third and fourth instar nymphs[2] were each allowed to feed, one per seedling alfalfa plant, for a period of 8 hours, at which time they were removed with a malathion spray. The 8-hour period was selected to insure adequate incidence of disease without the complication of the possible extremes (0 and 100% incidence). Each trial consisted of 10 plants per treatment and was replicated four times, so that each instar group was tested on 40 plants. Readings for yellow vein-banding symptoms were made on the third and fourth days following exposure.

A second experiment was done similarly, but using only single first and second instar nymphs, each on 100 plants

[2] The third and fourth instar nymphs were pooled, since separation, in the absence of tedious rearing techniques, was difficult and when done resulted in a collection of recently molted third instar nymphs and old fourth instar nymphs which introduced a bias which could not be readily overcome and which would have an unknown effect on the results.

Table 2.—**Results of trials to determine if single nymphs of *Therioaphis maculata* varied according to age (instar) in their ability to induce toxic symptoms on Caliverde alfalfa seedlings.**

Nymphal Instar	Number of Plants		Per Cent Positive
	Tested[a]	Positive[b]	
Experiment I			
1st	40	21	52.5
2nd	40	14	35.0
3rd & 4th	40	24	60.0
Experiment II			
1st	100	40	40.0
2nd	100	37	37.0

[a] The access time was 8 hours. In experiment I—10 plants per treatment, 4 replications. In experiment II—20 plants per treatment, 5 replications.

[b] Plants read a minimum of 2 successive days beginning 72 hours after initial infestation. Only those plants with symptoms on two or more occasions recorded as positive.

to give further data on the possible separation of these two instars as to relative toxicity. The results of the two experiments are given in table 2. The results confirmed the hypothesis that the nymphs in the later instars are more toxic than those in the earlier instars. Evidence of this statement can be supported statistically (pooled results, X^2 (adjusted) = 4.94, d.f. 1, $p = < 0.05$. The evidence for a difference between the first and second instars, however, was not statistically supportable at an acceptable 5% level of probability (X^2 (adjusted) = 1.21, d.f. 1, $p = > 0.20$).

The increased toxicity associated with the later nymphal instars may be a function of increase in size and the amount of feeding activity. However, this is somewhat negated by the fact that the first instar nymphs, the smallest of all used, were at least equal to, in fact tended to exceed the larger individuals of the second instar.

Variability in Disease Incidence.—Some plants do not display toxic symptoms, even after long exposure to individual spotted alfalfa aphids, which suggests that either the aphids vary in their toxicity or the plants in their susceptibility, or both. That alfalfa plants vary in susceptibility both qualitatively and quantitatively is used as the basis of selection for resistance among susceptible varieties (Harvey & Hackerott 1956), and was stressed by Paschke & Sylvester (1957) as a limiting factor in tests to measure damage.

On the other hand Carter (1950) demonstrated that a toxiniferous insect may inherit its toxic propensity when he showed that the toxicity of nymphs of the psyllid, *Paratrioza cockerelli* (Sulc.) was influenced by the demonstrated toxicity of their parents as nymphs. Thus experiments were conducted to determine how much, if any, of the observed variability in the results of tests with Caliverde alfalfa seedlings and *T. maculata* was due to intrinsic differences in individual aphids and selected clones of this species.

In the first experiment, 15 adult apterae were placed singly on each of 15 alfalfa seedlings and transferred daily to new plants on each of 5 successive days. At the time of transfer the progeny which had been deposited were removed. The plants were checked for disease symptoms on the second and third days after removal of the aphids. The number of single aphids whose feeding resulted in symptoms developing for at least 1 day on 0 to all of the five successive plants upon which each aphid had fed are given in figure 2 (upper). The histogram had a mode of 4 and a mean of 3.4 plants affected for each aphid. However, there is no indication as to whether the distribution was due to variation in aphid toxicity or variation in host plant susceptibility, or both, but it did serve as an expectancy model.

The second experiment, conducted in a similar manner, used 23 aphids and the individuals responsible for the two extremes of the distribution were kept for further tests. Figure 2 (center) shows the frequency distribution for these aphids. This distribution again had a mode of 4 but a slight increase in the mean value (3.6) positive out of 5 possible for each aphid. The aphids numbered 2, 8, and 14, each of whose feeding had resulted in symptoms on all five of the plants on which they had been tested (hereafter referred to as the "high" group) and the aphids numbered 11, 16, and 19, each of whose feeding had resulted in symptoms occurring on only 1 or 2 of the 5 plants (hereafter referred to as the "low" group) were selected for further tests. Each was placed on a separate plant and caged to prevent cross-infestation. Using some of the progeny from each of the six clones, a third experiment was done to test the hypothesis that progeny from the "low" group would be less toxic than those from the "high" group.

Four late instar nymphs from each of the six clones were selected and each was placed on an alfalfa seedling and subsequently transferred to a new plant each day for 5 days. Disease readings were made as in the earlier trials. Table 3 gives the proportions of plants affected by each clone and figure 2 (lower) the frequency distribution histograms of numbers of plants affected. In this experiment the 24-hour feeding period resulted in such a high incidence of disease in both groups that any difference which may have existed might have been masked. The modal difference (5 for the "high" clones versus 4 for the "low") of the histograms however, did not deny the hypothesis and warranted repetition of the experiment using a shorter exposure time in an attempt to increase sensitivity

Table 3.—**Results of trials to determine if the ability of *Therioaphis maculata* to produce a high or low incidence of disease symptoms on Caliverde alfalfa seedlings was inherited.**[a]

Status[b] of Parent							
Low				High			
Clone Number	Plants		Per Cent Positive	Clone Number	Plants		Per Cent Positive
	Tested[c]	Positive[d]			Tested	Positive	
Test access time 24 hours							
11	20	17	85	2	20	18	90
16	18	16	89	8	18	18	100
19	20	17	85	14	20	17	85
Test access time 5 hours							
11	20	19	95	2	19	13	68
16	18	10	55	8	20	14	70
19	20	18	90	14	20	10	50

[a] Four progeny from each clone were tested singly on each of five successive test plants.

[b] Low indicates that feeding of parent for 24 hours on each of five plants resulted in symptoms on two or less of them, while high indicates symptoms resulted on all five plants tested.

[c] Variations from 20 in this column are due to the elimination of data of those plants on which no aphid was found at the end of the feeding period.

[d] Plants examined on two occasions—those displaying yellow vein-banding symptoms on one or both observations were considered positive.

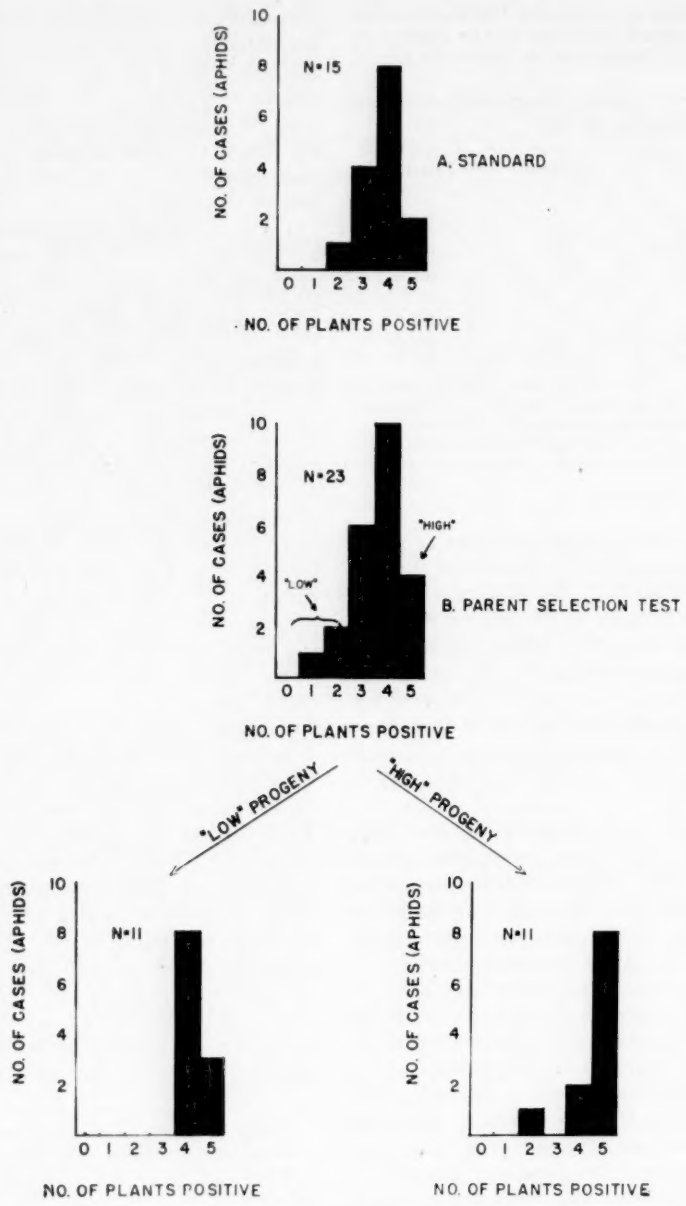

FIG. 2.—Histograms of results of tests designed to select and demonstrate possible inherent tendency of certain clones of *Therioaphis maculata* to vary in the ability to induce yellow vein-banding symptoms in Caliverde alfalfa seedlings.

of the test. This was done with a 5-hour access period, and the results are given in table 3. In this case feeding by the individuals of the "low" group resulted in a greater number of plants with toxic symptoms, quite conclusively indicating that differences, if any, which may have occurred among individual aphids in the ability to produce toxic effects were not inherited. Denial of the hypothesis indicated that most of the variability encountered was due to differences in host plant susceptibility rather than to differences among the insects.

Feeding and Toxin Injection.—The stylets of hemipterous, plant-feeding insects are usually surrounded by a proteinaceous sheath when in host plant tissue. Such sheaths were observed as early as 1878 (Prillieux 1878). Since then they have been described for a variety of Heteroptera and Homoptera, and various views have been set forth as to the origin and composition of them. Smith (1933) reviewed a number of reports concerning the feeding tracts of suctorial insects, and also conducted microchemical color tests on sheaths produced in plant tissue by the potato leafhopper, *Empoasca fabae* (Harris), and the three-cornered alfalfa hopper, *Spissistilus festina* (Say). In these experiments all tests for plant reserves showed response, and cell wall substances were negative, except for pectin compounds; whereas the tests for proteins and amino acids indicated the sheath material to be largely proteinaceous.

Horsfall (1923) considered the sheath of aphids to be composed of calcium pectate and protein material. Bradley (1952) described the sheath of the green peach

aphid as composed of saliva in the form of a colorless material flowing from the tip of the stylets as they advanced through a cell, and which rapidly set to a gel. In addition to the supposition that viruses and the knowledge that radioactive metallic ions (Hamilton 1935, Day & Irzykiewica 1953) can be acquired through feeding and subsequently translocated to the saliva, recent experiments by Nuorteva (1955) demonstrated that a protease (papain) could be inbibed from synthetic nutrient fluid by a heteropterous bug and transferred in active form to the salivary glands. This led him to conclude that at least some of the toxic substances in the salivary glands of toxiniferous insects may originate from the host plants and thus are not produced solely by the insects. Further tests by this worker (1946a) indicated an absence of plant growth stimulating substance in the salivary glands of seven species of insects but growth inhibiting substances were found. Whether or not such materials would be present in the salivary sheath was not indicated.

In an attempt to learn something of the stylet sheath formation by *T. maculata*, adult apterae were allowed to feed through a plastic membrane[3] into water to produce sheaths. When cells made of a plastic membrane stretched over a plastic ring containing water were placed on a slide, penetration through the membrane of the stylets and formation of the salivary sheath could be observed with a compound microscope. When the aphids withdrew their stylets, the sheath remained attached to the membrane, which could be placed on a glass slide, dried and heated slightly to aid in fixation. Since the plastic, but not the sheaths, was soluble in ethyl alcohol, an alcohol rinse left the sheath on the slide which could then be mounted in balsam and studied at higher magnification. Stylets of both *T. maculata* and *Myzus persicae* have been prepared in this manner, and figure 3 is a photomicrograph of the salivary sheaths of these two species. Both species of aphids, from relatively distant genera, one toxiniferous, the other an important relatively nontoxic species on herbaceous hosts but an important vector of plant viruses, produce sheaths. The sheaths are similar, but distinct. That such a sheath can be formed in the absence of plant tissue indicates that at least an important portion of the sheaths observed in plant tissue preparations are of insect origin, although reaction, and/or combination, with plant substance is not excluded.

Since saliva is utilized in sheath formation in rather copious amounts, the question arises whether the toxic principle associated with the feeding of *T. maculata* is introduced principally at the time of initiation of feeding when the sheath is laid down, or whether it is continually introduced during the entire feeding period.

Carter (1950) observed that tomato psyllid nymphs make about one puncture per individual in 2½ hours compared to about 1½ tracks per individual in 24 hours. Since the toxic effect of 24 hours of feeding by two individuals was greater than that produced by 2½ hours of feeding by three nymphs, he concluded that the toxin of this species was likely to be introduced slowly over the whole course of feeding, and not limited to the period of sheath formation.

To test such a hypothesis with *T. maculata*, an experiment was done using late instar nymphs feeding for either 1 or 5 hours with some left to feed without artificial interruption, and some artificially interrupted periodically to insure at least five insertions and therefore the formation of at least five salivary sheaths during the feeding period. A total of five replications of six combinations, five plants per combination, were done. The results are given in table 4. Again only those plants displaying yellow vein-banding symptoms on at least two or three observation occasions (24 hours apart) were recorded as positive.

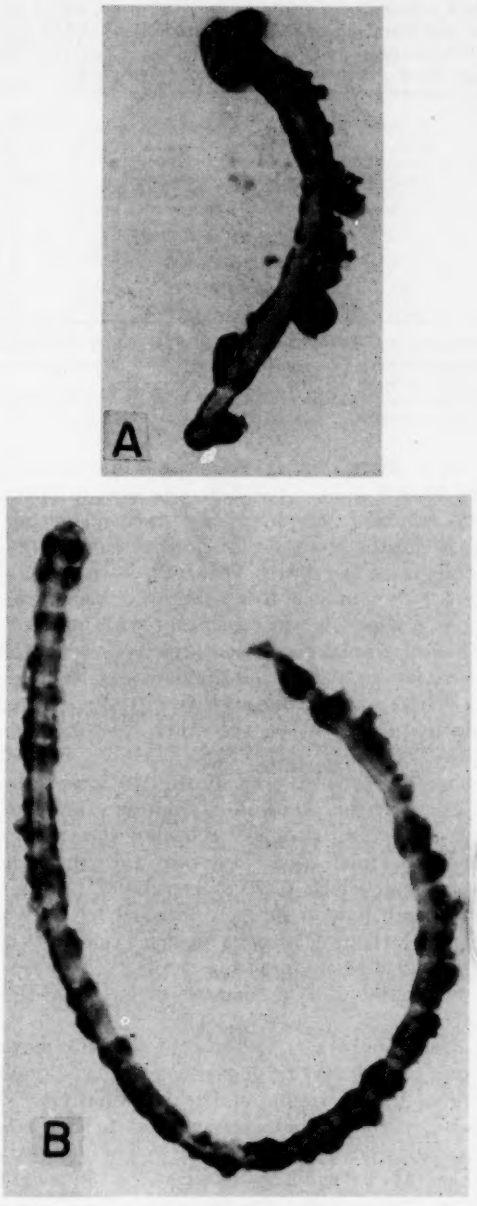

FIG. 3.—Photomicrographs of salivary sheaths produced by (A) *Therioaphis maculata* (Buckton) and (B) *Myzus persicae* (Sulzer) fed through a plastic membrane into water (×1600).

The results indicated that the liberation of toxin as measured by disease incidence was a constant and somewhat additive function of feeding time (8 positive out of 50 tested for 1 hour feeding versus 31 positive out of 50

[3] The membrane was made from one wall of a bubble blown with the plastic material "Aladdin Balunes" produced by Aladdin Sales Co., Jersey City 7, New Jersey.

Table 4.—Results of trials to determine the effect of the number and duration of feeding penetrations made by late instar *Therioaphis maculata* nymphs on Caliverde alfalfa seedlings on the incidence of systemic symptoms.

No. Aphids per Plant	Total Access Time per Aphid (Hours)	Type of Feeding permitted During Access Time[a]	Minimum Number of Penetrations Made	Results[b]	Ratio of Symptom Incidence to Hours of Access Time
1	1	continuous	1	3/25	3.0
1	1	discontinuous	5	5/25	5.0
5	1	continuous	5	18/25	3.6
1	5	continuous	1	15/25	3.0
1	5	discontinuous	5	16/25	3.2
5	5	continuous	5	18/25	0.7

[a] In continuous type of feeding aphid was left undistrubed during access period, in discontinuous, it was interrupted five times at somewhat equally spaced time intervals.

[b] Ratios in columns are, numerator-number of plants with symptoms; denominator-number tested.

tested for 5 hours feeding with single insects, X^2 (adjusted) = 10, d.f. 1, $p = <0.01$). It was evident also that toxin introduction was somewhat independent of the number of sheaths produced (18 positive out of 50 tested for a minimum of one sheath, versus 21 positive out of 50 possible, for a minimum of five sheaths with single insects). Evidently *T. maculata* was constantly injecting the toxic principle both during the period of active sheath formation (penetration) and during the period of feeding, and the loss of time spent on one activity was compensated by the gain in time spent on the other. The findings are similar to those reported by Carter (1950).

DISCUSSION.—The results indicate that after a threshold quantity of toxin needed for symptom expression has been introduced, with additional feeding the toxemia incidence increases at a constant rate until it reaches a plateau reflecting host plant susceptibility. Although a salivary sheath is more actively secreted by the aphids during penetration, the toxemia incidence does not appear to be dependent solely upon the number of penetrations made, but rather it is a function of the total feeding activity cycle.

The results do little to indicate the ultimate nature of the toxin. That it may be destructive to the chloroplasts is indicated by the results of Diehl & Chatters (1956), and that it is destructive to, or inhibitory to their production, is evidenced by the systemic yellow vein-banding symptoms. That it is translocated and is diffusible from the veins and veinlets into the surrounding mesophyll tissue is also indicated by the nature of the symptoms, but that its effects are temporary and reversible is indicated by the limited systemic action in the absence of the insects and the tendency for the affected tissues to recover.

Nuorteva (1956) reports that salivary protease occurrence can be correlated with the protein needs of *Miris dolabratus* L., and is highest at the time of egg formation. Since the late instar nymphs and adults of *T. maculata* are the most toxic of the various instars, a relationship of this nature also may exist here. Very desirable, however, would be the isolation and identification of the toxin itself. Methods of toxin collection attempted by the authors have included crushing aphids in Hoagland's solution, feeding aphids on cut stems through which a glass wick passed, and collecting alfalfa juice into which aphids had fed through membranes. However, before chemical analyses of such material would be meaningful, a bioassay for the presence of the toxin still needs to be developed. Various methods of introducing possible toxic materials with nutrient solutions, wicks, and puncture inoculations have thus far failed to give reproducible results. If on the other hand, as suggested by Nuorteva (1956a), the symptoms result from a toxin formed partially by host plant derivatives, the production, liberation, and stability of which is dependent upon the relative intact plant tissue environment, isolation will be even more difficult than is now apparent under the relatively simple hypothesis that the toxin is solely of insect origin.

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Investigations on Control of the Bronze Birch Borer and the Flatheaded Apple Tree Borer[1]

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ABSTRACT

Investigations in control of the bronze birch borer, *Agrilus anxius* Gory, and the flatheaded apple tree borer, *Chrysobothris femorata* (Olivier), were conducted at the Ohio Agricultural Experiment Station during 1956 and 1957. Several of the organophosphates and various concentrations of DDT were applied to the respective host trees. Parathion was consistently effective in the three tests in which it was used.

The results with DDT were erratic, but the data indicate that one application of a relatively strong concentration of DDT combined with a liquid latex or polyethylene on the trunks of newly transplanted shade trees may be adequate for the control of the flatheaded apple tree borer.

During the growing seasons of 1956 and 1957, tests were conducted at the Ohio Agricultural Experiment Station in control of two shade tree borers, the bronze birch borer, *Agrilus anxius* Gory, and the flatheaded apple tree borer, *Chrysobothris femorata* (Oliv.). Both are members of the family Buprestidae. The bronze birch borer is a severe pest in Ohio, particularly on paper birch. The damage caused to paper birch has been sufficient to curtail greatly the number of trees planted as ornamental trees in Ohio. The flatheaded apple tree borer is primarily a pest of newly transplanted or weak trees, but attacks most species of shade trees grown in Ohio.

BRONZE BIRCH BORER.—The bronze birch borer has been reported attacking almost all species of birch and poplar. However, Barter & Brown (1949) found that the species which attacks poplar in Canada is the bronze poplar borer, *Agrilus liragus* (Barter & Brown). In this investigation only white birch, *Betula alba*, and paper birch, *Betula papyrifera*, were used, because they are the species of birch most often found in ornamental plantings in Ohio.

Damage and Life Cycle.—The female deposits eggs in cracks and crevasses of the bark in the crown of the tree. These hatch in 10 to 11 days, and the larvae burrow into the cambium, often girdling the tree. The following spring the adults begin to emerge during the last week in May in northeastern Ohio, and the emergence period continues about 6 weeks. The females begin to oviposit about 8 days after emergence. The peak of adult activity, and therefore oviposition, occurs between the middle of June and the middle of July. The crowns of heavily infested trees present a stagheaded appearance, and many ridges in the bark circle the branches where the larvae have formed burrows. As the top of a tree dies the insects continue to attack successively lower branches. The length of time that an infested tree will survive varies with its age and vigor, but death usually occurs in 3 to 5 years.

Control.—In 1956, 35 birch trees of the white and paper varieties were used in studies on the biology and control of the borer. The trees ranged in condition from apparently healthy and uninfested to heavily infested and partially dead. They were situated on soil that was level to steeply sloping. Some of the trees were exposed to full sunlight, some partially shaded, and some almost completely shaded. An insecticide test was set up using five treatments with seven replications on single tree plots. Using a John Bean power sprayer the insecticides were applied to all the trunks and branches to the run-off point in all tests. The treatments applied are listed in table 1. The initial application of all treatments was made the last week in May to coincide with adult emergence. All treatments except No. 1 were applied again 20 days later, and No. 3 received a third application 20 days after the second.

Table 1.—Bronze birch borer control experiments, 1956.

Treatment No.	Materials Used	Gallons Used in 100 Gal.	No. of Applications	Condition of Trees in 1957	
				Deteriorated	No Change or Improved
1	DDT 25% EM	2	1	0	7
2	DDT 25% EM	1	2	1	6
3	DDT 25% EM	1	3	1	6
4	Parathion 25% EM	0.5	2	0	7
5	Untreated	—	0	6	1

After the treatments had been applied in 1956, notes were taken describing the condition of the trees. The amount and condition of the foliage and the character of the bark were noted. The following system was used in classifying the foliage: good, crown wilted, crown dead, and $\frac{1}{2}$ to $\frac{2}{3}$ dead. Ridges resulting from larval burrowing and adult emergence holes were described as numerous or light. The summation of the results of these observations is shown in table 1.

The treatments used in 1956 were repeated in 1957 except that parathion was used as a wettable powder instead of an emulsion concentrate. Also two additional treatments were included as shown in table 2. The first application was made on May 31 and, where more than one application was made, the interval between applications was approximately 30 days.

In September 1957, 41 linear feet of birch of approximately the same bark area from each treatment was dissected and the larvae counted. The data are presented in table 2.

The results of the work of two seasons indicate that all treatments reduced the borer populations. Treatment 4 in both 1956 and 1957 (parathion) and treatment 6 in 1957 (malathion) appeared to be most effective. The results with DDT were too variable to permit evaluation, but the data show no evidence that two or three applications were more effective than one. Most of the 103 larvae recorded in treatment 3, table 2, were from one tree. The variation in borer populations is apparently due to variations in tree vigor. In general, trees in a low state of vigor harbor more borers than healthy trees.

An observation program was set up in which the adult

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Table 2.—Bronze birch borer control experiments, 1957.

Treatment No.	Materials	Amount in 100 Gallons	No. of Applications	Larvae per 41 Linear Feet of Birch
1	DDT 25% EM	2 gal.	1	7
2	DDT 25% EM	1 gal.	2	41
3	DDT 25% EM	1 gal.	3	103
4	Parathion 15% WP	6.67 lb.	2	3
5	Untreated	—	—	192
6	Malathion 57% EM+ DDT 25% EM	2 gal. 1.5 gal.	1	2
7	DDT 50% WP+ polyethylene	5 lb. 5 gal.	1	17

Table 3.—Flatheaded apple tree borer treatments, 1957.

Treatment No.	Materials	Amount in 100 Gallons	No. of Applications	No. of Larvae in 13 Trees
1	DDT 25% EM	3 gal.	1	19
2	DDT 25% EM+ polyethylene	3 gal. 5 gal.	1	1
3	DDT 25% EM	1 gal.	2	2
4	Parathion 15% WP	6.7 lb.	2	0
5	Thimet 48% WP	5 lb.	1	12
6	Untreated	—	—	20
7	DDT 50% WP+ polyethylene	5 lb. 5 gal.	1	2[a]

[a] Only seven trees used.

beetles found on the trees during a specific period of time were collected periodically. Biweekly, seven check and seven treated trees were each observed for approximately 2 minutes. It is perhaps significant that during the two seasons in which observations were made, 135 adult beetles were taken from the seven check trees whereas not a single beetle was taken from any of the treated trees.

FLATHEADED APPLE TREE BORER.—*Tests on Shade Trees.*—The flatheaded apple tree borer has been recorded attacking a wide range of hosts. The hosts used in this test consisted of 80 maples of the red, silver, Norway, and Crimson King varieties, 10 white birch, 10 dogwood, and 20 Moraine thornless honey locust. These were nursery stock, transplanted in April, 1956, at the Ohio Agricultural Experiment Station. The base diameters ranged from 1 to 3 inches. They were situated on level to slightly sloping terrain and all were exposed to full sunlight.

A special procedure was used as a means of attracting the beetles. Quart cans were attached to the lower ends of 12 freshly cut apple stems or boles 30 inches in length. These boles were attached to trees at random in the planting, and the cans were filled with water. It was found that by supplying the cut apple boles with water, they could be kept fresh and moist for a considerable time, some of them sending out leaf sprouts. These boles were not treated with insecticides.

Control.—The treatments used in 1956 were applied to the trunk and large branches only, using a 5-gallon capacity John Bean power sprayer. The treatments, each replicated 18 times (20 trees in check series), were applied on May 25. The application date was adjusted to the emergence period of the beetles, which began about the last week of May in Ohio.

All of the trees were examined for borer injury in the fall of 1956, but only an occasional tree was found to be injured. It seemed probable that adult beetles were either killed or repelled by the insecticides. However, 10 trees died during the fall and winter months, and when the bark was removed from the dead trees, borers were found in seven of them. A treatment which included parathion was the only one in which all trees survived.

The treatments used in 1956 were repeated in 1957 and a seventh treatment was included (table 3). The sprays were again applied with a 5-gallon capacity John Bean sprayer. The first application was made on June 3, and treatments 3 and 4 were applied a second time on July 9. Each treatment was applied on 14 trees distributed at random, and 16 trees were not treated. A liquid polyethylene was used in treatments 2 and 7. This material was included in an attempt to augment the residual effect of the toxicant used, to seal up potential egg sites, and to make the trees less attractive to the ovipositing beetles.

In June of 1957 some of the roots were severed with a spade in order to simulate transplanting and thereby make the trees more attractive to the beetles. Freshly cut apple boles in quart cans were again used. In this instance 24 boles were used, and they were placed in the shade tree planting in the soil near the bases of the trees in a crisscross pattern across the planting.

In September the bark was removed from 13 trees of each treatment and the larvae counted, except for treatment No. 7 in which only seven trees were stripped. The results are presented in table 3.

It is interesting to note that the parathion treatment was again outstanding. Thimet, as used in this experiment, seemed relatively ineffective, and the DDT treatments were again variable. However, the two treatments in which DDT was used in combination with the liquid polyethylene compound both appeared effective.

Tests with Apple Boles—McDaniel (1933) noted that wind-blown trees often served as breeding places for *C. femorata*. Also Johnson & Fenton (1939) showed that because eggs are deposited readily in freshly cut apple and walnut wood, boles can be used effectively in borer control experiments. With this in mind a test was arranged using freshly cut apple boles as host material in 1956. The boles were 35 inches in length and the diameters were such as would allow them to fit in quart motor oil cans. The ends of the boles were placed in the cans and attached with nails. The cans were placed in the soil, with the boles upright, and filled with water. There were five treatments replicated four times, including the four checks. The treatments were randomized. All insecticides were applied to the run-off point on June 14, 1956 using an electric atomizer. The results were taken in late September by removing the bark from the boles and counting the borers. The data are presented in table 4. Treatment

Table 4.—Cut apple bole treatments, 1956.

Treatment No.	Materials	Gallons Used in 100 Gal.	No. of Larvae in 4 Boles
1	DDT 25% EM	4	2
2	DDT 25% EM+ Latex	4 32	0
3	Parathion 25% EM	0.5	2
4	DDT 25% EM	1	6
5	Untreated	—	14

number 2 applied at the rate of 4 gallons of the 25% DDT emulsion with 32 gallons of liquid latex in sufficient water to make 100 gallons gave complete control, whereas the same concentration of DDT without the latex was equal in degree of control to ½ gallon of 25% parathion emulsion. The results of this test could not be analyzed statistically and so the test was repeated in 1957.

In 1957, 60 cut apple boles were used. They were placed in an area adjacent to the shade tree planting. Unfortunately, this area was shaded by tall trees on the eastern, southern, and western sides for all except about 3 hours of the day. When the boles were dissected, not a single larva was found, whereas in those boles which had been placed in the shade tree planting to attract the beetles, an average of seven larvae per bole were found. The only explanation seemed to be that the shade tree planting was exposed to full sunlight throughout the day. This seems to indicate that sunlight is an important factor in the attractiveness of a tree for this beetle.

DISCUSSION.—The outstanding feature in the results of the tests in control of the two species of borers was the consistently good result obtained with parathion. Although the parathion treatment resulted in complete control in only one test, it effected acceptable control in all cases. Perhaps more consistent control would have been expected with DDT because of its greater stability and longer lasting qualities. However, the results correspond with those of E. H. Smith (1951) on control of the peach tree borer in New York. The tests on control of the flatheaded apple tree borer indicate that the effectiveness of DDT may be improved by the addition of a liquid latex or polyethylene compound. Although the data are not conclusive, they indicate that one application of such a combination on the trunks of newly transplanted trees may be adequate for the control of the flatheaded apple tree borer.

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Tests on Lindane and Aldrin Vapors in the Control of Adult Mushroom Flies[1]

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ABSTRACT

Experiments were conducted in Pennsylvania and Maryland on the control of mushroom flies, chiefly the sciarid fly, *Bradysia fenestralis* (Zett.), and the phorid fly, *Megaselia agarici* Lintner, with lindane and aldrin vapors blown into mushroom houses with electric fans. Vapors were formed by passing the air streams through Fiberglas air-conditioning filters previously treated with the insecticides. The treatment killed adult flies without leaving excess residues or adversely affecting the flavor or aroma of the mushrooms.

Lindane vapor from treated filters mounted in front of an electric fan was found by Sullivan (1951) to kill house flies and mosquitoes. In 1951 and 1952 experiments were conducted in Pennsylvania and Maryland to determine the value of lindane and aldrin vapors in the control of adult mushroom flies, chiefly the sciarid, *Bradysia fenestralis* (Zett.), and the phorid, *Megaselia agarici* Lintner.[4] The vapors were dispersed by passing an air stream through a filter previously treated with the insecticide. An acetone solution of the insecticide (1:7) was applied with a hand sprayer to both sides of a Fiberglas air-conditioning filter. The acetone soon evaporated, leaving a coating of crystals, to give a long-lasting source of insecticide vapor. The filter was fastened with wire to three points on the guard of an electric fan of the type used in homes and mushroom houses.

Preliminary tests at Beltsville, Md., in experimental mushroom houses 8,000 cubic feet in size indicated that mushrooms would tolerate a 4-hour treatment when 50 grams of the insecticide was used on a single filter mounted on a standard 16-inch mushroom-house fan and that the vapors would kill the adult mushroom flies that were present.

EXPERIMENT 1.—This test was performed in May 1951 in two standard 16,000-cubic-foot houses at Toughkenamon, Pa., one of which was used as an untreated check. Two filters 16 by 25 by 1 inches were each sprayed with 100 grams of lindane dissolved in acetone and then attached to 16-inch fans. The fans were placed on the floor at opposite ends of the middle walk and faced the ceiling at the center of the house. They were operated from 11:45 A.M. to 3:30 P.M. on May 10, from 7 to 10 A.M. on May 11, and from 7 to 10 A.M. on May 12. Before the start of each treatment, sheets of white paper about 10 by 15 inches were placed at 12 locations throughout each house to catch the flies as they dropped from the air and died. The number of flies taken on these papers

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[2] Retired June, 1953.

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[4] Identified by Allan Stone and W. W. Wirth of the Entomology Research Division, U.S.D.A.

Table 1.—Numbers of mushroom flies killed by lindane and aldrin vapors and collected on 12 papers 10 by 15 inches. Toughkenamon, Pa., 1951.

Experiment	Date	Temperature (° F.)	Treated House		Untreated House	
			Sciarids	Phorids	Sciarids	Phorids
		Lindane				
1	May 10	56	92	29	0	0
	11	56	86	28	19	7
	12	57	117	32	0	0
2	June 20	67	59	30	0	0
	21	68	86	31	8	2
		Aldrin				
3	June 20	68	61	47	0	1
	21	70	78	113	1	0

within 1 hour after the fans were cut off are given in table 1.

On May 10 no flies were observed about the overhead lights after the treatment. However, before the treatments on the 11th and 12th, a few flies were seen near the lights and on the under side of the beds and near the door cracks. Apparently they had emerged from the beds since the previous treatment. The flies in the untreated check house on May 11 may have been killed by the lindane vapors that escaped from the adjacent treated house.

EXPERIMENT 2.—In experiment 2 another mushroom house at Toughkenamon identical to that in experiment 1 was treated with lindane in a similar manner on June 19, 20, and 21, 1951, except that one of the two filters was 20 by 25 by 1 inches and attached to a standard heavy-duty mushroom-type fan. The house was treated 4 hours on each day. There was little mortality at the end of the June 19 treatment. The data in table 1, taken after the treatments on June 20 and 21, show better results. There was a continual emergence of flies from the compost and mushrooms in the beds. Apparently the lindane vapors killed only those flies on the wing during the treatments and did not penetrate into the compost.

EXPERIMENT 3.—This test was conducted in a 28,500-cubic-foot mushroom house at West Chester, Pa., in June 1951 to test the toxicity of aldrin vapor. The equipment and procedures were the same as in experiment 2 except that the house was treated with aldrin vapors 4 hours daily. Since the house had two middle walks and there were no electric outlets on the north end, a fan was placed on the floor at the south end of each middle walk. An adjacent house was used as an untreated check. The dosage of aldrin was 100 grams on the larger filter, as with lindane, but only 50 grams on the smaller one. The results, shown in table 1, were very similar to those with lindane. The aldrin vapor appeared to be effective against both species of mushroom flies.

EXPERIMENT 4.—This test was conducted at Reisterstown, Md., in 1952 in two heavily infested standard mushroom houses of 16,000 cubic foot capacity. The equipment and procedures were the same as in experiment 2 except that the two fans were operated on a time switch 4 hours daily from May 15 to 29. There was a gradual reduction in fly population in the treated house. By May 29 very few adults were seen on the wing or on the floor, but the walks and bed surfaces were strewn with thousands of dead flies. Large numbers of living flies were observed as usual in a nearby untreated house. Experiment 4 was repeated in nearby infested houses on June 5 to 9, with similar results.

EXPERIMENT 5.—This test was conducted at West Chester, Pa., in 1952, in a large mushroom house 100 feet long and containing 28,000 cubic feet of space. Two lindane-treated filters mounted on fans operated on a time switch were used; otherwise the procedure was the same as in the previous tests. The vapors were dispersed in the house for 4 hours daily from October 23 to 27, and November 2 to 6. The results were in agreement with those of the earlier tests. The sciarids were found to be more susceptible to the vapors than the phorids. On November 6, while the house was being treated, samples of air were aspirated from both the treated and the untreated houses at 64° F. and analyzed (Hornstein & Sullivan 1953). There was an average of 0.024 microgram of lindane per liter of air after correction for the reading in the untreated house. This value is considerably below the lindane concentration in air at saturation conditions at this temperature, which is approximately 0.3 microgram per liter. The exposed surfaces in these houses were probably very absorbent to lindane vapors. The lindane concentration in the air cannot be regarded as indicative of the lindane concentrations in the soil and mushrooms. No analyses were made of the soil during the treatment. Samples of mushrooms from the treated house were analyzed by the Schechter and Hornstein method (1952).[5] There was a residue of 0.4 p.p.m. of lindane, which is well below the established tolerance of 10 p.p.m. In informal taste tests no adverse effects were detected in the flavor or aroma of the cooked mushrooms.

The results of these tests indicate that the adult population of the two most important flies that attack mushrooms can be greatly reduced. For best results it is necessary to repeat treatments for at least 4 consecutive days, since large numbers of adults continue to emerge daily from the compost and mushrooms of the producing beds and soon build up a heavy population. Although both lindane and aldrin vapors are toxic to mushroom flies when used in the manner described above, further studies are needed under the varying conditions met in commercial production in various parts of the United States. This technique should also be tested with some of the recently developed pesticides that are more easily volatilized, such as the phosphorus compounds.

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Control of Mosquito Larvae in Log Ponds in Oregon[1]

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ABSTRACT

DDT, heptachlor, and malathion emulsions at 1, 3, and 6 pounds of technical material per surface acre were used in tests for the control of mosquito larvae in log ponds in the general vicinity of Corvallis, Oreg. Protection against reinfestation ranged from 3 to 6 weeks for DDT, 5 to 10 weeks for heptachlor, and 2½ to 6 weeks for malathion. *Culex pipiens* L. was the dominant species in ponds in June and July; *C. stigmatosoma* Dyar predominated in August, September, and October. Except in the cooler months from October through May, *Culex tarsalis* Coq., *C. territans* Walker, and *Culiseta incidens* (Thompson) were represented by relatively small percentages.

Log ponds are an integral part of the Oregon lumber industry and, since modern logging trucks have made it possible for sawmills to become concentrated near permanent population centers, most communities in the western part of the State contain at least one pond, and some have as many as a dozen in their immediate vicinity. These ponds, ranging from one-fourth to more than 100 acres, have a great potential for mosquito production, and their present distribution has caused the mosquitoes from them to become important health hazards and nuisances. Since the lumber industry throughout the Pacific Northwest uses log ponds, mosquito control in the ponds is a regional problem.

Several mosquito-abatement districts operate within western Oregon, and the control of larvae in log ponds is one of their main problems. This problem seems certain to increase, since larvae of *Culex tarsalis* Coq. have developed resistance to DDT in ponds in at least one area of the State (Eddy *et al.* 1958).

Stage *et al.* (1952) recommended 3 quarts per acre (about 0.2 p.p.m.) of a 25% DDT emulsifiable concentrate for the control of larvae in log ponds. Hoffman & Yates (1956) reported that slightly better initial kills and a longer residual effectiveness were obtained with a DDT emulsion than with a 50% DDT wettable-powder spray. They stated also that "surface oils provided a good initial kill but reinfestation occurred more rapidly than with the emulsions." In a series of field tests designed to compare various dosages per acre of DDT and EPN in emulsions, they found little difference in the performance of the two insecticides. In laboratory tests with log pond and tap water at 60° to 70° F., their results suggested a negative temperature coefficient for DDT in either medium, a slight positive temperature coefficient for EPN in tap water, but not in pond water, and some reduction of effectiveness of both insecticides in log pond water, possibly due to suspended matter or an unidentified chemical condition.

Because of the need for more information on the mosquito problem in log ponds, a series of tests was conducted in 1957 with commercially available insecticides in the vicinity of Corvallis, Oreg.

MATERIALS AND METHODS.—The effectiveness of DDT, heptachlor, and malathion in emulsions was compared at rates of 1, 3, and 6 pounds of technical material per surface acre. Tests were run in duplicate at the two lower rates, but only one pond was treated with each at the 6-pound rate. Ponds were randomized and the materials applied in 1% emulsions with a power sprayer. Since good coverage with ground equipment could not be obtained on large ponds, ponds of less than 1 acre were generally used. Populations were estimated by a minimum of 20 dips. Protection was judged to have ended when reinfesting larvae had developed to the fourth instar.

Table 1.—Weeks' protection against mosquito larvae in log ponds in western Oregon with three insecticides at three dosages per acre.

INSECTICIDE	DOSAGE (LB.)		
	1	3	6
DDT	3	4.5	6
Heptachlor	5	8	10+
Malathion	2.5	5	6

Turbidity of the ponds was measured with a Secchi disc and their pH values with a Model G Beckman glass electrode pH meter. The stability of each emulsion in distilled water was compared with its stability in the pond water in which it was used. Only the DDT formulation was found to be unstable in pond water. A formulation containing 65% of xylene, 10% of Triton X-100, and 25% of DDT was therefore prepared in the laboratory.

RESULTS.—Control results are given in table 1. With each insecticide the residual effectiveness increased with the dosage. The turbidity and pH values are presented in table 2. Control results could not be correlated with the turbidity readings or with the pH values. Several lumbermen stated that logs from some species of trees discolor water more than others, and general observations indicated this was true. Secchi disc readings are, therefore,

Table 2.—Turbidity and pH values of 15 log ponds in western Oregon in 1957.

POND NO.	VISIBILITY IN INCHES OF SECCHI DISC	pH VALUES
1	6.0	6.6
2	4.0	6.8
3	6.5	7.4
4	6.5	5.7
5	5.5	6.3
6	5.0	6.6
7	8.5	6.8
8	5.5	7.0
9	5.5	6.1
10	13.0	5.8
11	4.5	6.8
12	4.0	7.0
13	8.5	6.6
14	5.0	5.2
15	4.5	5.0

[1] Accepted for publication September 15, 1958.

[2] Leland Brown assisted in these investigations.

Table 3.—Per cent of different species of mosquito larvae collected from log ponds in western Oregon from July 1957 through June 1958.

Month	*Culiseta incidens*	*Culex*			
		stigmatosoma	*pipiens*	*tarsalis*	*territans*
July	12	25	61	2	0
August	6	62	14	18	0
September	3	91	4	2	0
October	30	65	1	4	0
November	97	3	0	0	0
December–March	99	1[a]	0	0	0
April	77	0	0	17	6
May	49	10	13	27	1
June	6	26	55	13	0

[a] December collection only.

not always a true measure of a pond's turbidity. Although average pond depth varied somewhat, control results could not be correlated with average depth.

DISCUSSION.—Most of the treated ponds were close to untreated ones, and constantly contained many viable egg masses. Consequently there was an immediate heavy reinfestation when insecticidal effectiveness ceased, which characteristically occurred abruptly.

Adult emergence from untreated ponds was high until midOctober. The monthly species trend from the beginning of the treatment period through June 1958 is presented in table 3. *Culex pipiens* L., the chief pest species present, predominated in June and July, but decreased steadily during the succeeding months and constituted only 1% of the October total. *Culex stigmatosoma*[3] Dyar was the dominant species in August, September, and October. Except in the cooler months from October through May, *Culex territans* (Walker), *C. tarsalis*, and *Culiseta incidens* (Thom.) were represented by relatively small percentages. Although *Culex tarsalis* was present in small percentages during most of the year, the total number of potential disease-carrying individuals is evident from the fact that peak larval populations in the ponds ranged from slightly less than 100 to more than 800 larvae per dip.

In the laboratory *Culex tarsalis* and *stigmatosoma*, and *Culiseta incidens* were reared to the pupal stage about as quickly on finely ground Douglas-fir cambium, on moss from Douglas-fir logs, and on lichens from the same source as on a standard high-protein diet, which indicates that log ponds contain a rich and constantly renewed source of larval nutrients. However, by the same method evidence was obtained that fresh cedar cambium is slightly toxic to larvae, which suggests that the species of trees that constitute the log source may be one of the factors that influence a pond's ability to produce larvae.

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[3] The designation, *C. stigmatosoma*, reverted to *C. peus* Speiser after this paper was in type.

Factors Affecting Transmission of Bean Yellow Mosaic Virus[1]

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ABSTRACT

Tests were made to determine the effect of different species of plants as sources of virus and as healthy indicator plants on the transmission of bean yellow mosaic virus by the aphids *Myzus persicae* (Sulz.) and *Macrosiphum pisi* (Harris). Source plants caused significant differences in transmission by both aphids. Broad bean (*Vicia faba* L.) was consistently a better virus source plant than alsike clover (*Trifolium hybridum* L.), Alaska pea (*Pisum sativum* L.) and Dwarf Horticultural bean (*Phaseolus vulgaris* L.). There were no differences between these four species in their susceptibility to aphid inoculation with the virus.

The relative susceptibility of five bean varieties to inoculation with bean yellow mosaic virus by the green peach aphid and the pea aphid was determined. These five horticultural varieties were not equally susceptible to aphid inoculation. Relative susceptibility varied with the aphid species used and with environmental conditions.

The bean yellow mosaic virus infects a number of legume species (Goodchild 1956). This paper presents the results of tests to compare some of these plants as sources of virus for aphid transmission and to compare their relative susceptibility to aphid inoculation. Since this work was completed, Corbett (1958) has reported differences between lupine species as sources of bean yellow mosaic virus in insect transmission.

MATERIALS AND METHODS.—The bean yellow mosaic virus used in this work was described by Swenson (1954). The virus was obtained in July, 1956 and was maintained in broad bean (*Vicia faba* L.) by transmission with the

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green peach aphid (*Myzus persicae* (Sulz.)). Two aphid species were used in the tests reported herein. The green peach aphid was reared on broccoli (*Brassica oleracea* L.) and the pea aphid (*Macrosiphum pisi* (Harris)) was reared on broad bean. Aphids were starved for a period of 30 minutes or more. They were then transferred individually to infected source plants by the camel's-hair brush technique. Feeding periods on the infected source plants were observed through a hand lens and timed with a stop watch. In the first experiment reported, green peach aphids were allowed a naturally terminated feeding period of 2 minutes or less on the infected source plant. In all subsequent experiments, these feeding periods were naturally terminated and 10 to 30 seconds in duration. Aphids were then transferred to healthy test plants, at the rate of one aphid per plant in all tests, where they were left for 1 hour or more before the plants were fumigated with nicotine and returned to the greenhouse. Plants used in each experiment were selected for uniformity. Greenhouse temperatures were controlled at 75±5° F. during the day and 65±5° F. at night. Treatments were randomized in each replicate.

EFFECT OF VIRUS SOURCE PLANT AND TEST PLANT COMBINATIONS ON TRANSMISSION BY THE GREEN PEACH APHID AND THE PEA APHID.—Broad bean, Dwarf Horticultural bean, alsike clover and Alaska pea were used in all possible combinations as virus source plants and healthy test plants for green peach aphid transmission of the virus. Thus there were 16 combinations of virus source plants and healthy test plants in each replicate. There were 30 replicates.

Broad bean was a better source of virus than the other three species of plants (difference significant at 1% level). The four species of plants were about the same in susceptibility to virus inoculation (table 1).

Sixty-three per cent of the aphids used terminated feeding on the infected source plants in 10 to 30 seconds. The minimum time necessary for aphids to acquire the virus was between 6 and 10 seconds.

Broad bean, Alaska pea, alsike clover and Dwarf Horticultural bean were used as virus source plants for pea aphid vectors in the second experiment in this series. Dwarf Horticultural beans were used as healthy test plants. In each replicate of the experiment, one aphid was transferred from an infected plant of each of the four source species to each of five test plants. Thus there was a total of 20 test plants per replicate. There were 14 replicates.

Broad bean was the best source of virus for pea aphid transmission (difference significant at 5% level) (table 1). Comparison of results of the first two experiments shows that broad bean was the best source of virus for both the green peach aphid and the pea aphid. The order of efficiency of the four species of virus source plants was different for the two aphids.

A third experiment was designed to test the two aphid species under comparative conditions with the various source plants. Broad bean, alsike clover and Alaska pea were used as virus source plants for green peach aphid and pea aphid transmission. Dwarf Horticultural beans were used as healthy test plants. The three species of source plants and the two aphid species were used in all possible combinations. This resulted in six test plants in each replicate and the experiment was replicated 30 times.

Table 1.—Effect of four species of virus source plants and test plants on transmission of bean yellow mosaic virus by *Myzus persicae* (Sulz.) and *Macrosiphum pisi* (Harris).[a]

	1[b]	2	3	
Broad Bean	44/120[c]	27/120	14/70	37/30 13/30
Alsike Clover	35/120	38/120	1/70	17/30 7/30
Alaska Pea	26/120	34/120	10/70	13/30 7/30
Dwarf Horticultural	22/120	28/120	3/70	

[a] Based on three experiments.
[b] 1, Transmission by *Myzus persicae* when source plants and test plants respectively; 2, Test plants infected by *Macrosipaum pisi;* 3, Test plants infected by *persicae* and *pisi*, respectively.
[c] Per cent infection over number of plants inoculated.

Broad bean was the best source of virus for both aphids (difference significant at 1% level) (table 1).

RELATIVE SUSCEPTIBILITY OF BEAN VARIETIES TO VIRUS INOCULATION BY APHIDS.—The bean varieties Dwarf Horticultural, Top Crop, Bountiful, Black Valentine and Stringless Blue Lake were tested for susceptibility to virus inoculation by the green peach aphid. Alsike clover was the virus source plant in replicates 1–20; broad bean was the virus source plant in replicates 21–29. Five beans of each variety were inoculated in each replicate.

The bean varieties differed in susceptibility to virus inoculation, Stringless Blue Lake being the most susceptible (difference significant at 1% level) (table 2).

Dwarf Horticultural, Top Crop, Bountiful, Black Valentine and Stringless Blue Lake beans were then tested for susceptibility to virus inoculation by the pea aphid. Five plants of each variety were inoculated in each replicate and there were eight replicates. Broad bean was used as a virus source plant.

Stringless Blue Lake was the variety most susceptible to virus inoculation by the pea aphid (difference significant at 5% level) (table 2).

Comparison of the results of experiments one and two (table 2) shows that Stringless Blue Lake was the variety most susceptible to inoculation by both aphids. The order of susceptibility of the varieties was not the same for both aphids.

The third experiment in this series was designed to test the susceptibility of two bean varieties to inoculation by the green peach aphid and the pea aphid under comparative experimental conditions.

Stringless Blue Lake and Dwarf Horticultural beans

Table 2.—Susceptibility of five bean varieties to bean yellow mosaic virus inoculation by *Myzus persicae* and *Macrosiphum pisi*.[a]

	1[b]	2	3	
Blue Lake	45/145[c]	30/40	43/30	23/30
Dwarf Horticultural	20/145	8/40	43/30	10/30
Black Valentine	13/145	20/40		
Top Crop	12/145	10/40		
Bountiful	12/145	8/40		

[a] Based on three experiments.
[b] 1, Transmission by *Myzus persicae;* 2, Transmission by *Macrosiphum pisi;* 3, Transmission by *persicae* and *pisi*, respectively.
[c] Per cent infection over number of plants inoculated.

were used as healthy test plants. Broad bean was the source of virus. One aphid of each species was transferred from the virus source plant to one plant of each of the test species in each replicate. Thus there were four test plants in each replicate and there were 30 replicates.

The two varieties were equally susceptible to virus inoculation under the conditions of this experiment (table 2). The only significant difference was that the green peach aphid was more effective than the pea aphid.

DISCUSSION.—The rate of transmission of both persistent and non-persistent aphid-borne viruses and leafhopper-borne viruses can be affected by the virus source plant used (Severin 1939, Freitag & Severin 1945, Bawden & Kassanis 1946, Kirkpatrick & Ross 1952, Kassanis 1952, Simons 1955, 1957, Bagnall & Bradley 1958, Corbett 1958). This is true also for bean yellow mosaic virus since in the experiments reported in this paper, broad bean was consistently a better source of virus than the other source plants tested with both the green peach aphid and the pea aphid.

Various plants may show differences in relative susceptibility to inoculation of both persistent and nonpersistent viruses and leafhopper-borne viruses (Watson 1938, Severin & Tompkins 1948, Kassanis 1952, Kirkpatrick & Ross 1952, Frazier & Thomas 1953, Jensen 1957, Sylvester & Simons 1951, Simons 1955, Bagnall & Bradley 1958).

The results of the experiments reported in this paper show that there were no differences in the relative susceptibility of the four species of plants tested. Differences in susceptibility to virus inoculation were found in experiments with five bean varieties. Stringless Blue Lake was the variety most susceptible to virus inoculation in the first two tests of varietal susceptibility. There was no difference in susceptibility between Stringless Blue Lake and Dwarf Horticultural beans in the third experiment. The first of these experiments was made in November and December, the second in June and the third in August (table 2). The most striking difference was in the results obtained with the Dwarf Horticultural and Stringless Blue Lake bean varieties when exposed to inoculation by *M. persicae* in November-December tests as compared with the tests made in August. The results obtained in these experiments appeared to depend upon the environmental conditions under which the plants were grown.

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Action of Repellents on Mosquitoes Feeding Through Treated Membranes or on Treated Blood[1]

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ABSTRACT

A method for testing repellents against *Aedes aegypti* (L.) feeding on citrated blood through an artificial membrane is described. Repellents were applied at various concentrations on the membrane or in the blood, and the concentration required to give 50% repellency was determined. The percentage of mosquitoes that fed was affected by the time between anesthesia by carbon dioxide or cold and feeding. The second factor was not a critical one. When applied on the membrane or in the blood, diethyltoluamide was the strongest repellent, followed by dimethyl phthalate and ethyl hexanediol. Dimethyl phthalate applied on the membrane probably acted as a vapor repellent only. There were indications that blood acted as a vapor stimulant in feeding. Rabbits injected intravenously with diethyltoluamide did not repel the mosquitoes, nor did the blood taken from injected rabbits and offered through a membrane. It was concluded that the repellent was removed from the blood very rapidly.

The different procedures employed for testing mosquito repellents have contributed to a wide variation in results. The method used in the screening tests at the Orlando, Fla., laboratory was adapted from one developed by Granett (1940). A standard amount of the candidate repellent (usually 1 ml.) is applied to the forearm, which is then exposed for 3 minutes at approximately 30-minute intervals to caged *Aedes aegypti* (L.) or at 20-minute intervals to caged *Anopheles quadrimaculatus* Say. Effectiveness is usually based on the time between treatment and the first confirmed bite (a bite followed by another in the same or the subsequent exposure period). Sometimes the experimental repellent is compared directly with a standard repellent on the opposite arm of the same subject (Gilbert *et al.* 1953, Travis *et al.* 1949).

Where quick and rough evaluation of a large number of materials is needed, this method is satisfactory. It is even suitable for more critical comparisons when used in properly designed experiments with an adequate number of replications. It has the advantage of evaluating the repellents under conditions close to those of actual use. However, when precise, noncomparative determinations are necessary, this method, as well as similar methods used by various investigators, is inadequate because it is subject to large variations caused by known factors, as pointed out by Travis (1950) and Dethier (1956). The duration of effectiveness differs greatly on different individuals. Even the same individual may show wide variations from day to day. Whether the test animals are men, canaries (Wasicky *et al.* 1949), mice (Eddy & McGregor 1949), rabbits (Starnes & Granett 1953), guinea pigs (Kasman *et al.* 1953), or cattle, the repellent is being tested on a variable background. The amount of sweat on the skin and the biting rate of the mosquitoes affect the repellent time. Comparisons computed on time to the first or even to the fifth bite are based on the ends of a normal distribution curve, statistically the least significant portion and hence subject to the greatest variability.

The use of an olfactometer is not entirely satisfactory for this type of test, as pointed out by Kasman *et al.* (1953), since an olfactometer measures only the effect of the repellent vapor without regard to the surface from which the vapor arises or the effect of tactile repellency.

A method of testing repellents against mosquitoes feeding through an artificial membrane has therefore been developed. The advantages of such a method are: (a) The experiments are carried out under standard conditions with no living animals or human background. (b) The repellent is used at a range of concentrations and the one giving 50% repellency (the most sensitive point in the curve) can thus be determined. (c) The repellents can be tested not only on the membrane but also in the blood and between membranes. (d) Mosquitoes from the same stock cage can be used at the same time—*i.e.*, under exactly the same conditions—to compare a repellent at different concentrations or various repellents at the same concentration. (e) The amount of repellent required is very small, an important item in investigations with rare and expensive compounds.

Several artificial membranes have been used in the feeding of mosquitoes and other arthropods on blood. Recently Tarshis (1958) found Silverlight membrane,[4] a commercial product prepared from the outermost layer of ox caecum, satisfactory for feeding several species of arthropods. This membrane was therefore used in these tests except in some of those described in the section on factors affecting feeding.

EQUIPMENT, METHODS, AND MATERIALS.—After several preliminary experiments, the following standardized techniques were adopted. Certain variations from these methods will be found in the section on "Factors Affecting Feeding."

The equipment consisted of cylindrical screen cages 18 inches long and $2\frac{5}{8}$ inches in diameter to hold the mosquitoes, glass cylinders $2\frac{1}{2}$ inches in diameter and 2 inches deep to hold the blood, and an incubator to maintain the blood at a constant temperature (fig. 1). At various intervals prior to testing, 6-day-old mosquitoes (*Aedes aegypti*) were anesthetized with carbon dioxide and about 100 were placed in each cage. The cages, usually nine in the same incubator in each test, were connected to holes in the floor of the incubator, below which they were suspended, and the top of each cage was closed temporarily with a cardboard disk. Except as specified in the tests in the section on "Factors Affecting Feeding," the mosquitoes were anesthetized and caged 24 hours prior to testing, and were given honey and water.

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[2] Formerly Wolfinshon, visiting entomologist from the Israeli Institute for Biological Research, Ness-Ziona, Israel.

[3] Many members of the Orlando laboratory staff, especially I. H. Gilbert and H. K. Gouck, assisted in these studies.

[4] Manufactured by Julius Schmid, Inc., New York N. Y

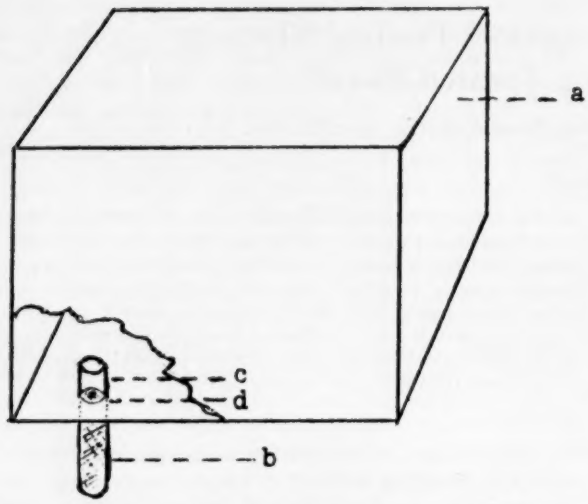

FIG. 1.—Diagram of feeding apparatus: (a) Incubator, (b) cage containing mosquitoes, (c) glass cylinder containing blood, (d) membrane.

The bottoms of the glass cylinders were covered with taut Silverlight membrane, which becomes firmly attached when applied to moistened glass. Blood was obtained from the abattoir, citrated by adding 1% of a 50% solution of sodium citrate, and kept in the refrigerator at 5° C. for periods up to 5 days. At the time of testing, 20 ml. of the blood, warmed to 40°, was poured into each of the cylinders, which were placed inside the incubator over the holes above the cages containing the mosquitoes, the cardboards covering the cages having been removed. In some tests repellents were applied to the surface of the membrane, and in others they were emulsified in the blood. The incubator containing the cylinders of blood was maintained at 40° but owing to evaporation the temperature of the blood itself was usually about 36° while the mosquitoes were exposed to room temperature of 28° and a relative humidity of 70 to 80%. After the mosquitoes had been given an opportunity to feed on the blood for 3 hours, they were again anesthetized, transferred to test tubes and killed with ether, and the per cent that had fed was determined.

The repellents used were dimethyl phthalate, ethyl hexanediol, and diethyltoluamide.

Per cent repellency was obtained from the formula $100 (N-T) \div N$, in which N is the average per cent fed on the control (untreated membrane or blood) and T the average per cent fed on the treated membrane or blood.

FACTORS AFFECTING FEEDING.—Before the method of testing was standardized as described above, several variations were tested to select the one that would induce the highest percentage of mosquitoes to feed. In these experiments the mosquitoes were anesthetized with carbon dioxide 2 to 4 hours before blood was offered. For each factor studied the mosquitoes were from the same stock cage, anesthetized at the same time, and tested under exactly the same conditions with controls.

The following variations did not show any significant difference in the per cent of mosquitoes that fed: (a) Keeping the mosquitoes in a cylinder closed at the bottom with a Silverlight membrane and immersing the bottom in blood kept at 34° C. by a water bath, as described by Tarshis (1956). (b) Fresh blood and blood kept in the refrigerator at 5° for 1 to 5 days, but from the sixth day on a noticeable reduction in feeding was observed. (c) Blood kept at 32° or 36° during feeding. (d) Morning or afternoon feeding. (e) Blood kept under pressure by compressing air in the cylinders above it; this modification was considered because the blood in the body is under pressure.

In these experiments the average feeding per cage was $64.3 \pm 2.4\%$. The time that elapsed between anesthetizing the mosquitoes and the start of feeding was next investigated, and was found to be an important factor, as shown in table 1. The differences in the per cent that fed when tested $\frac{1}{2}$, 4, and 7 hours after anesthesia were significant ($P > 0.05$); the larger the recovery period, the larger the percentage that fed. Also the variations, as seen from the standard errors, were the smallest after 7 hours and the largest after $\frac{1}{2}$ hour. A similar difference in feeding was obtained between mosquitoes knocked out by a 15-minute exposure to cold (5° C.) $\frac{1}{2}$ hour and 7 hours prior to feeding.

In the succeeding experiments the mosquitoes were anesthetized and placed in the feeding cages 24 hours before the blood was offered. There was no difference between mosquitoes starved during this period and those allowed to feed on honey and water. More than 90% fed in both cases. In the following experiments the mosquitoes were allowed to feed on honey and water during the 24-hour period before testing.

There was a highly significant difference ($P > 0.01$) between blood kept at room temperature of 28° C. at the time of feeding ($62.7 \pm 3.7\%$ fed in 9 cages, a total of 1,411 mosquitoes) and blood kept at 36° ($93.3 \pm 1.7\%$ fed in 4 cages, a total of 630 mosquitoes).

REPELLENTS APPLIED TO MEMBRANE.—The effect of various dosages of the repellents applied to the outer surface of the membrane was determined. Mosquitoes from a single stock cage were placed in nine feeding cages, usually two cages for testing each of three dosages and three cages as controls. At first the repellent was diluted in ethyl alcohol and equal volumes of various dilutions were applied on the membrane. However, the repellent remained on the membrane in the form of droplets after the alcohol evaporated. The addition of spreading and wetting agents improved the spreading somewhat, but the results were still unsatisfactory. Full-strength repellent was therefore applied to the membrane to give dosages of 1.3, 2.6, and 5.2 mg. per square inch.

Table 1.—Effect of time from anesthesia with carbon dioxide or from knockout by cold (5° C.) to start of feeding on the per cent of mosquitoes that fed.

	ANESTHESIA (6 REPLICATIONS)		COLD (8 REPLICATIONS)	
HOURS	Total number	Per Cent Fed	Total Number	Per Cent Fed
0.5	869	53.0 ± 6.2	984	75.5 ± 5.7
4	686	73.1 ± 3.0	—	—
7	690	92.1 ± 1.0	836	91.3 ± 0.8

The results are given in table 2. Diethyltoluamide was the most effective repellent and dimethyl phthalate second. The differences between diethyltoluamide and ethyl hexanediol were highly significant at all the dosages, between diethyltoluamide and dimethyl phthalate at all but the highest, and between dimethyl phthalate and ethyl hexanediol at all but the lowest.

The amounts required to repel 50%, as obtained from a repellency-dosage regression line plotted on log-probit paper, were 1.7 mg. for diethyltoluamide, 2.0 mg. for dimethyl phthalate, and 4.8 mg. for ethyl hexanediol.

REPELLENT BETWEEN TWO MEMBRANES OR ON A MEMBRANE COVERED WITH SCREEN.—Frings & Hamrum (1950) have shown that *A. aegypti* possesses tarsal receptors for an acceptable food material (sucrose solution). Wallis (1954) and Hudson (1956) have shown that this mosquito, as well as other species, can distinguish low concentrations (0.085M) of salt solutions in their oviposition sites and that the sensitivity is present in the tarsal segments. The possession of tarsal chemoreceptors that enable insects to distinguish acceptable from nonacceptable materials in solution has been shown by various investigators (Dethier 1951, 1955, Frings & Frings 1949). However, there is no proof as yet that tarsal chemoreceptors, although acting as food rejectors, cause insects to be repelled from a treated surface. Bar-Zeev and Dethier (unpublished) have found that a saturated solution of potassium chloride or 0.1 M sulfuric acid does not repel *A. aegypti*.

The following experiments presented an opportunity to study the effect of a repellent on the tarsal chemoreceptors. Covering a treated membrane with an untreated membrane or screen would enable the mosquitoes to land on an untreated surface to feed. If the per cent that fed increased significantly under such conditions, it could be assumed that tarsal chemoreceptors were probably involved in repelling the mosquitoes. On the other hand, if there was no effect, tarsal chemoreceptors were probably not involved (at least at the concentration of repellent used) and the mosquitoes were repelled by vapor only.

In one experiment dimethyl phthalate was applied to the membrane, and either (a) another membrane was placed over and in direct contract with it or (b) the membrane was covered with a wire screen (18 mesh and 0.8 mm. thick). As shown in table 3, when the repellent was applied between two membranes, the per cent repellency was significantly greater at 2.6 mg. per square inch, and the difference was highly significant at 1.3 mg., than in the tests with the repellent on the outer surface of a single membrane (table 2). At 5.2 mg. per square inch the difference was not significant. On a membrane covered with a wire screen the repellency was greater (the difference was highly significant) than on a membrane without a screen, although the mosquitoes fed readily through a screen in the control.

In another experiment a repellent was placed between two membranes separated by a similar wire screen so that the first membrane, which was covered with the repellent, would not touch the second. However, only 1 of 225 mosquitoes in the control lots without a repellent fed under such conditions.

REPELLENTS IN THE BLOOD.—Tests were made with the repellents emulsified in the blood at several concentrations. Ten per cent of Tween 80 (polyoxyalkylene derivative of sorbitan monooleate) was added as an emulsifying agent. The results are given in table 4. Diethyltoluamide

Table 2.—Effect of repellents applied to the membrane on feeding by mosquitoes.

Repellent (Mg./Sq. In.)	Number of Replicates	Total Number of Mosquitoes	Average Per Cent Fed	Per Cent Repellency
		Diethyltoluamide		
1.3	11	1,365	60.3±4.6	34.9
2.6	10	1,321	18.5±4.8	80.0
5.2	10	1,239	7.5±2.3	91.9
Control	13	1,548	92.7±1.6	—
		Dimethyl phthalate		
1.3	10	1,459	72.8±2.5	23.3
2.6	10	1,447	34.1±4.3	64.1
5.2	10	1,518	6.8±0.9	92.8
Control	14	2,164	94.9±1.3	—
		Ethyl hexanediol		
1.3	10	1,081	79.6±3.7	15.1
2.6	10	948	72.3±3.9	22.9
5.2	10	1,164	43.4±5.5	53.7
Control	15	1,830	93.8±1.1	—

Table 3.—Effect of dimethyl phthalate between two membranes and on a membrane covered with a screen on feeding by mosquitoes.

Repellent (Mg./Sq. In.)	Number of Replicates	Total Number of Mosquitoes	Average Per Cent Fed	Per Cent Repellency
		Between two membranes		
1.3	10	1,041	51.3±6.1	44.2
2.6	10	1,234	20.6±2.8	77.6
5.2	10	1,206	4.5±1.5	95.1
Control (2 membranes)	15	1,755	91.9±1.4	—
		Covered with a screen		
2.6	4	477	2.0±0.8	97.9
Control (with screen)	4	539	94.0±1.1	—

Table 4.—Effect of repellents emulsified in the blood on feeding by mosquitoes.

Per Cent Repellent	Number of Replicates	Total Number of Mosquitoes	Average Per Cent Fed	Per Cent Repellency
		Diethyltoluamide		
0.1	10	1,003	0.9±0.3	99.0
.033	11	1,188	58.7±3.9	36.9
.01	10	1,174	86.2±2.7	7.4
.001	10	1,183	90.2±2.9	3.1
Control	15	1,948	93.1±1.2	—
		Dimethyl phthalate		
.1	10	745	19.1±3.5	79.2
.033	11	1,291	85.3±2.2	7.3
.01	10	725	80.0±2.6	13.0
.001	10	759	85.5±2.6	7.1
Control	9	643	92.0±2.5	—
		Ethyl hexanediol		
.1	4	483	86.7±1.2	6.6
.033	4	441	91.8±2.1	1.1
.01	4	450	89.9±1.4	3.1
Control	6	758	92.8±1.6	—

was more repellent than dimethyl phthalate, the difference being highly significant at the two highest concentrations. Ethyl hexanediol had little repellent effect at these concentrations. The R-50 as obtained from the repellency-dosage regression line was 0.040% for diethyltoluamide and 0.089% for dimethyl phthalate. The highest concentration of ethyl hexanediol did not cause 50% repellency.

The high degree of repellency caused by a low concentration of diethyltoluamide or dimethyl phthalate in the blood was rather surprising, and suggested that the material might be passing through the membrane and exerting its repellent effect from the surface. An experiment was therefore carried out to determine whether the membrane picked up enough repellent from the blood to repel the mosquitoes. Twenty milliliters of blood containing 0.1% of diethyltoluamide was kept in each of five cylinders at 36° C. for 3 hours, and then the blood was removed and the inside of each cylinder was rinsed with a little water. Twenty milliliters of new blood, without the repellent, was then poured into each cylinder, and the feeding on the blood in these cylinders and four check cylinders that had not contained treated blood was determined. Of 528 mosquitoes exposed to the five cylinders from which treated blood had been replaced with untreated blood, 94.7 ± 2.3% fed, and 93.7 ± 0.43% of 461 mosquitoes fed on the check cylinders. It is therefore apparent that the repellent effect previously observed was exerted by the repellent in the blood, not by that absorbed in or on the surface of the membrane.

INJECTION OF DIETHYLTOLUAMIDE INTO BLOOD OF ANIMALS.—The fact that diethyltoluamide at a low concentration (0.1%) repelled 99% of the mosquitoes suggested the possibility of using it as a systemic repellent. Kingscote (1952) introduced a number of repellents into the stomachs of experimental animals to see whether they would reach the blood stream and eventually be given off from the skin, and thus repel insect pests. The animals did not develop a repellent action against *Aedes aegypti* by this method of administration. In our experiments intranvenous injection was tried.

Diethyltoluamide containing 10% of Tween 80 was slowly injected, during 10 minutes, into the ear veins of three rabbits. Enough repellent was used to produce a concentration of 0.4% in the blood, based on reference data indicating that the blood comprises 6.2% of the weight of the rabbit. At intervals of ¼, ½, 1, 2, 4, and 8 hours after the injection a cage containing about 100 mosquitoes was placed on the shaved belly of the rabbit for 15 minutes. The cage was held in feeding position with a clamp attached to a ringstand. Practically all the mosquitoes fed on the injected rabbits at each interval, as they did on three noninjected ones.

Other rabbits were injected with 0.4% of diethyltoluamide in the same way, and samples of blood were taken out of the rabbit at various intervals after the injection (¼, ½, 1, 2, and 4 hours). Five rabbits were injected, one for each time interval. This blood was then tested for repellency to mosquitoes feeding through a membrane. In these tests the cylinders containing the blood were only 0.94 inch in inside diameter, to reduce the amount of blood needed. More than 90% of the mosquitoes fed on the blood of the injected rabbits and on blood taken from five noninjected rabbits, an indication that the diethyltoluamide was destroyed or removed from the blood system practically as fast as it was introduced.

DISCUSSION.—The method of feeding mosquitoes on citrated blood through a Silverlight membrane appeared to provide a satisfactory means of comparing the initial repellency of compounds applied to the surface of the membrane. The method was less satisfactory when the repellent was mixed with the blood, since only two of the three good practical repellents tested exerted any appreciable effect through the membrane. Differences between compounds were significant near the level of 50% repellency, but not always at higher or lower levels.

The action of the repellents on the surface of the membrane appears to be due to the vapor only, as the effectiveness of dimethyl phthalate was increased, not decreased, when a screen or a second membrane was placed over the treated membrane to serve as an untreated landing place. The extra mechanical difficulty of biting under these conditions may have added to the repellency, although the mosquitoes fed normally under these conditions when no repellent was present. The possible mode of action of the repellents in the blood is discussed in another paper (Bar-Zeev & Schmidt 1958).

Granett & Haynes (1945) found ethyl hexanediol to be more effective than dimethyl phthalate, whereas our experiments have shown the contrary to be true. The difference may be due to the fact that in our experiments the initial repellency was tested whereas in the others the length of the protection period was compared, although other differences in the techniques of testing cannot be overlooked. The initial repellency and the time of a repellent's effectiveness are obviously not necessarily related.

The quick removal of the repellent from the blood of an injected rabbit indicates the difficulties encountered in using systemic repellents.

The fact that when two membranes were separated by a screen only 1 of 225 mosquitoes in the control lots fed indicates that the mosquitoes will feed only through a membrane that is in close contract with blood. The distance between the two membranes (0.8 mm.) was much shorter than the length of the proboscis, which could easily reach the blood if probing were attempted. Although the membranes are semipermeable (Bar-Zeev & Schmidt 1958), the air space separating them may greatly reduce the concentration of volatile molecules from the blood reaching the mosquitoes, at least during the experimental period (3 hours), since many hours might be needed to achieve equilibrium in the air space between the two membranes. It is possible that stimulating materials from the blood are absorbed to the outer surface of the membrane and stimulate through contact chemoreceptors. However, since the mosquitoes fed on an untreated membrane covered with a screen as well as without a screen (see table 3), the blood probably acted as a stimulant through vapor. Although the temperature at the outer surface of the membrane is also greatly reduced because of the air between it and the heated blood, unheated blood still gave a high percentage of feeding (62.7 ± 3.7). Also there was no significant difference between the percentage of mosquitoes that fed when the blood was heated to 32° or 36° C. For these reasons

the difference in temperature does not seem of sufficient importance to account for the failure of the mosquitoes to feed.

The effectiveness of the blood due to constituents acting as vapor stimulants is in agreement with the results obtained by Burgess & Brown (1957), Reuter (1936), and Laarman (1955), but contrary to those of Howlett (1910), who found that simple exposure of blood was unattractive to species of Culicini. The difference may be due to the fact that a wet surface repels the mosquitoes by water vapor. Roth & Willis (1952) found that *A. aegypti* preferred drier air when presented with a choice between 75 and 100% relative humidity, and Thomson (1938) found that *Culex* avoided both high and low humidities. Therefore, blood exposed openly to mosquitoes might repel them because of the high relative humidity near the surface. However, Burgess & Brown and Reuter found blood to be more attractive than water. A difference in the relative humidity close to the surface of these materials may have been the main factor causing this difference in attractiveness. In the present investigation the blood was covered by a semipermeable membrane. This probably reduced the relative humidity at the surface, thus preventing the repellent effect of a high relative humidity and permitting stimulating materials in the blood to produce a favorable response.

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Action of a Repellent as Indicated by a Radioactive Tracer[1]

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ABSTRACT

P^{32}-phosphoric acid was used as a tracer in experiments with *Aedes aegypti* (L.) feeding through a membrane on citrated blood. When diethyltoluamide was present in the blood at a concentration that prevented the mosquitoes from engorging, the mosquitoes barely touched the membrane and did not insert their proboscises into the blood. When the concentration was lowered to one that allowed about half the mosquitoes to become engorged, those that did not feed spent slightly more time in contact with the membrane, and about a third of them inserted their proboscises into the blood but did not imbibe. It was concluded that most of the repellency was due to vapor action but at a low concentration contact chemoreceptors on the labella may have been involved.

Mosquito repellents applied to the skin have been reported to keep the mosquitoes at a distance by exerting vapor action (Sarkaria *et al.* 1951) or, in case of those with low volatility such as Indalone, acting presumably as contact repellents (Christophers 1947).

Field experience by many workers at the Orlando, Fla., laboratory has shown that most repellents hold mosquitoes at a short distance from the skin when freshly applied in adequate quantities, but that they gradually become less effective. As this happens the mosquitoes begin to land on the arms but leave almost instantly. As the number of landings increases, some mosquitoes remain longer on the skin, walking about or standing in one place but not attempting to probe. Later they touch the proboscis to the skin, as though about to bite, but do not actually probe. They may make such contacts in several places, and frequently they stop and rub the proboscis with the feet as though to clean it. Eventually they begin to bite.

This behavior suggests that the repellent is first active at a distance, but later only by contact, and that it repels by contact with the mouth parts at weaker concentrations than by contact with the tarsi. This appearance may be misleading, however, as it has not been demonstrated that different sets of chemoreceptors are involved. To determine whether the repellency was due solely to vapor action or whether contact chemoreceptors were involved, two experiments were conducted with diethyltoluamide added to blood.

The tests were made with yellow-fever mosquitoes (*Aedes aegypti* (L.)), and the procedures were the same as those in another study (Bar-Zeev and Smith 1958), except that smaller membrane-covered cylinders with less blood were used. Phosphoric acid labeled with P^{32} was added to the blood at the rate of 40 microcuries per milliliter, or applied to the surface of the membrane, and counts of the activity on the tarsi and proboscises were made to determine whether the mosquitoes had been in contact with the membrane or the blood.

To insure the detection of minute traces of blood on the proboscis, it was necessary to have highly radioactive blood. To keep the total activity at the minimum, the quantity of treated blood was reduced from that used by Bar-Zeev and Smith to 2 ml. in glass cylinders 0.94 inch in inside diameter. Preliminary tests showed that mosquitoes fed as readily from these smaller cylinders as from those used by Bar-Zeev and Smith. In the following experiments the blood in some cylinders contained 0.5% of diethyltoluamide which prevented all feeding, and that in others 0.065%, which allowed about half the mosquitoes to feed during the 3-hour exposure.

EXPERIMENT 1.—The first experiment was conducted to determine whether the mosquitoes were repelled without contact between the proboscis and blood at high and low concentrations of the repellent. Four groups of mosquitoes were used. Group I was allowed to feed on blood without repellent. The proboscises from 15 females that had engorged fully were counted for radioactivity to determine the amount to be expected in the proboscis of insects which had imbibed. To determine how much activity a proboscis would acquire if it barely touched the radioactive blood, proboscises from 15 other mosquitoes not preveiously offered blood (group II) were dissected and the outer sheaths (labia) removed. Each proboscis was cemented to the end of a wooden applicator stick. About five drops of blood were placed in a vial made of 5-mm. glass tubing, and the vial was held upright in a cork stopper. Under a dissecting microscope the proboscises were dipped into the radioactive blood, allowed to dry for a few minutes, and counted for radioactivity.

Mosquitoes in group III were offered radioactive blood treated with 0.5% of diethyltoluamide, and those in group IV blood with 0.065%. The proboscises from 15 unfed (repelled) mosquitoes in both groups were dissected and counted for radioactivity, to determine whether there had been contact with the blood, and if so whether the mosquitoes had started to imbibe.

The activity, in counts per minute, on the proboscises of individual mosquitoes that had fed on radioactive blood or been repelled by radioactive blood containing repellent, and on proboscises that had been quickly dipped in radioactive blood was as follows:

Group I (No Repellent)	*Group II (Proboscis Dipped in Blood)*	*Group III (Blood Containing 0.5% Repellent)*	*Group IV (Blood Containing 0.065% Repellent)*
206	10	0	0
239	11	0	0
265	12	0	0
316	12	0	0
358	16	0	1
363	19	0	3
375	25	0	4
377	25	0	5
415	31	0	6
510	34	0	7
511	35	1	7
515	35	1	8
556	43	2	8
619	58	5	12
768	240	5	14

Counts of 6 or less per minute were not considered to indicate that a proboscis had touched the blood, as the background count varied to this degree. Therefore, at

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0.5% diethyltoluamide the mosquitoes did not insert their proboscises into the blood, since none showed more than 5 counts per minute. At 0.065% about 6 of the 15 nonfed (repelled) mosquitoes did insert their proboscises but did not imbibe any blood, as the counts ranged from 7 to 14 per minute, about equal to the lowest of those obtained on dipped proboscises but far below those of fed mosquitoes.

EXPERIMENT 2.—The second experiment was conducted to determine whether the mosquitoes that do not insert their proboscises in the blood are also kept from landing on the membrane. The labeled phosphoric acid was diluted with equal parts of Carbowax 400 and spread on the membrane. One drop of Tween 80 was added to insure better spreading, and about two drops of red indicator to make the solution visible in order to check the evenness of the coating. Ten microliters of the solution containing 28 microcuries of P^{32} was spread on each membrane with a glass rod. Four groups of mosquitoes were used. Mosquitoes of group I were allowed to feed through the P^{32}-treated membrane on blood without repellent. The legs were then removed from 15 mosquitoes that had fully engorged and radioactivity counts were made to determine the range of activity that could be expected to be picked up on the legs of a mosquito while feeding to repletion. These data represented the maximum activity that could be expected on tarsi of mosquitoes landing on the membrane. The 15 mosquitoes in group II were not offered blood but were killed and held so that two to four legs of each mosquito touched the membrane lightly; the legs of each mosquito were then counted for radioactivity to provide an indication of the range of activity that could be expected to be picked up on the legs of mosquitoes that landed briefly on the membrane but were immediately repelled rather than remaining on the membrane to feed. Mosquitoes in group III were offered blood treated with 0.5% of diethyltoluamide and those in group IV blood with 0.065%. The legs from 15 unfed (repelled) mosquitoes in both groups were removed and counted for radioactivity, to determine how much contact there had been with the membrane by individual mosquitoes in each group before they were repelled.

The activity, in counts per minute, on the legs of individual mosquitoes that had fed on untreated blood, or been repelled by blood containing repellent, or on legs which had been lightly touched to the membrane, was as follows:

Group I (No Repellent)	*Group II (Legs Held on Membrane)*	*Group III (0.5% Repellent)*	*Group IV (0.065% Repellent*
1,384	7	5	4
1,601	7	17	11
1,737	8	27	24
1,882	9	28	32
2,069	9	31	33
2,306	9	32	54
2,585	10	33	59
2,758	12	51	69
2,957	13	52	103
3,167	14	57	126
3,923	14	60	133
4,358	17	63	140
4,443	19	64	142
4,729	27	84	363
5,835	31	255	427

The mosquities that were held with legs barely touching the membrane (group II) showed about half the activity of the mosquitoes that did not feed when exposed to 0.5% of diethyltoluamide (group III). This indicates that at this concentration practically all the mosquitoes just touched the membrane. At 0.065% of diethyltoluamide (group IV) about half the mosquitoes showed higher activity, indicating that they may have walked a little on the membrane before they were repelled, or that they landed several times. The legs of the fed mosquitoes (group I) showed very high activity.

The semipermeability of the membrane was also demonstrated in these experiments. When the membrane was coated with 28 microcuries of the labeled phosphoric acid, the blood that had been in contact with the opposite side for about 3 hours showed 11,700 counts per minute per microliter.

DISCUSSION.—If the mosquitoes in these experiments had not touched the membrane, it would have been proof that the repellency was due solely to vapor action. The fact that the mosquitoes barely touched the membrane at a high concentration (0.5%) of the repellent in the blood but did not insert their proboscises into the blood, may give the same indication—that molecules of the repellent, escaping from the blood and passing through the semipermeable membrane, acted on the olfactory chemoreceptors. The possibility that an effective amount of the repellent might have been accumulated, through absorption, on the outer surface of the membrane, and thus acted on contact chemoreceptors of the tarsi is contraindicated by the results of Bar-Zeev and Smith (1958), who showed that an effective amount of repellent did not accumulate on the surface of a membrane in contact with blood containing 0.1% of diethyltoluamide.

For the same reasons it seems unlikely that contact chemoreceptors on the tarsi were involved in the repellency at 0.065% of diethyltoluamide, even though some mosquitoes were not repelled until they had spent some time walking on the membrane. The more reasonable assumption is that at the lower concentration of repellent in the blood there was less vapor in the surrounding air, and the mosquitoes were repelled only after having come very close to the source, actually walking a little on the membrane.

Inasmuch as some mosquitoes inserted their proboscises into the blood but did not imbibe, contact chemoreceptors on the labella, shown to exist on *A. aegypti* (Frings & Hamrum 1950), might have been involved.

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Relation of Structure to Stability, Antiesterase Activity and Toxicity With Substituted-Vinyl Phosphate Insecticides[1]

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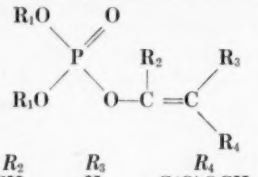
Abstract

Phosdrin, Shell OS-1808, OS-1836 and many related substituted-vinyl phosphates were found to be mixtures of geometrical isomerides which could be separated on chromatographic columns. The major component in each case was found to be the fraction first eluted from the column (alpha) and the less stable isomeride. The alpha isomer was also the most active as an insecticide and anticholinesterase agent. The exact isomeric configuration of the alpha and beta fractions from these substituted-vinyl phosphates is as yet uncertain. An impurity present in technical Phosdrin insecticide was found to be 0,0-dimethyl 1-carbomethoxy-1-chloro-1-propen-2-yl phosphate. Irradiation of either the alpha or beta OS-1808 fractions with ultra-violet light yielded a mixture of the components in a ratio of about 70% beta and 30% alpha fractions. The toxicity of the alpha fraction of OS-1808 was not affected by the beta component in ratios as high as 10 parts beta to 1 part alpha. Studies with 23 amino acids for efficiency in catalyzing the hydrolysis of alpha-Phosdrin, indicated that the most active were proline and hydroxyproline. Certain pyridine aldoxime methiodides were more effective catalysts than any of the amino acids at physiological hydrogen ion concentrations. Column pure alpha fractions from 23 substituted-vinyl phosphates were compared as to alkaline stability, anticholinesterase activity, and toxicity. Many substituents about the vinyl grouping were found to yield quite high insecticidal activity.

Substituted-vinyl phosphates are among the most active insecticides. A generalized structure for this type of compound and some insecticidal examples are as follows:



Compound	R_1	R_2	R_3	R_4
Phosdrin	CH_3O	CH_3	H	$C(O)OCH_3$
DDVP	CH_3O	H	Cl	Cl
OS-1836	C_2H_5O	H	H	Cl
OS-1808 and NC-13[4]	C_2H_5O	CH_3	H	$C(O)OC_2H_5$

The chemical and insecticidal properties of Phosdrin (*O,O*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate and DDVP (*O,O*-dimethyl 2,2-dichlorovinyl phosphate) have been intensively studies (Corey *et al.* 1953a, 1953b, Perkow *et al.* 1952, Perkow 1954, Ivy 1953, Mattson *et al.* 1955, Barthel *et al.* 1955, Arthur & Casida 1957, Casida *et al.* 1956).

The relation of structure and isomeric configuration of substituted-vinyl phosphates to stability, antiesterase activity and toxicity was further studied. A preliminary report on this investigation has been published (Casida 1955).

Methods and Results.—*Properties of Some Technical Substituted-Vinyl Phosphates.*—Certain chemical and biological properties were compared for Phosdrin, OS-1808 (*O,O*-diethyl 1-carbethoxy-1-propen-2-yl phosphate), OS-1836 (*O,O*-diethyl 2-chlorovinyl phosphate), and NC-13 ((*O,O*-diethyl 1-carbethoxy-1-propen-2-yl phosphate) prepared by condensation of sodium acetoacetate with diethyl chlorophosphate (Schrader 1952) and supplied by Fisons Pest Control Ltd., England). These properties are presented in table 1.

The time for loss of one-half the organophosphate by volatilization from filter paper was determined by impregnation of 1-inch squares of coarse filter paper with 1.0 mg. of the organophosphate in acetone. Following the evaporation of the acetone a zero hour sample was taken immediately and the remaining squares were held at 25 to 35° C. Papers were removed at various time intervals and the organophosphate extracted with chloroform and determined by total phosphorus analysis. The volatility half-life was shortest for OS-1836 and the longest with OS-1808 and NC-13.

Permanganate was used to determine the susceptibility of the vinyl group to oxidative attack. The reduction rate of 0.0004 *M.* potassium permanganate in acetone solution by 0.015 *M.* vinyl phosphate at 28° C. was measured with a spectrophotometer at 530 millimicrons. The half-life of the permanganate was determined in minutes. OS-1836 effected the most rapid and NC-13 the slowest reduction of the permanganate.

Table 1.—Properties of some technical substituted-vinyl phosphates.

Property	Phosdrin	OS-1808	OS-1836	NC-13
Stability and volatility				
Volatility half-life (hours)	12	32	2	32
Oxidative half-life (MnO_4^-) (min.)	10	10	4	14
Biological activity				
pI_{50}[a] crystalline chymotrypsin	1.8	2.5	5.2	1.9
Biological half-life in plants (AntiChE.) (hours)	17	17	3.2	8
LD_{50} (topical) female house fly (mg./kg.)	0.45	1.9	2.7	22.5
LD_{50} systemic to pea aphids (p.p.m.)	0.8	15	12	>100

[a] Negative logarithm of molar concentration of inhibitor resulting in 50% enzyme inhibition.

For chymotrypsin inhibition studies, the enzyme and inhibitor were incubated together overnight at 4° C. and then the residual esteratic activity determined manometrically with a tyrosine ethyl ester substrate (Casida *et al.* 1954). OS-1836 was much more effective under these

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[4] OS-1808: for relationship to NC-13 see table 2, footnote 1.

conditions than the other vinyl phosphates in inhibiting the esteratic activity of crystalline chymotrypsin.

The biological half-life of the four technical materials was measured in pea plants under greenhouse conditions. Young pea plants were allowed to absorb the insecticide through the roots for 24 hours from a 1:300 solution (OS-1808 and Phosdrin) or a 1:150 solution (OS-1836 and NC-13). They were then surface-washed and transplanted into sand. The anticholinesterase agents present in the foliage were determined immediately after transplanting and at eight subsequent time intervals up to 96 hours. The first biological half-life for the materials is shown in table 1. Much of the loss in this initial biological half-life was due to volatilization. The second and third biological half-lifes for each of the chemicals were found to be about 15 hours.

Contact toxicity to flies was determined by topical application of 4-day-old adult female *Musca domestica* L. The insecticide was applied in 1.3 microliters of acetone to the tip of the abdomen and 24-hour mortality counts were made on three replicates of 10 flies each (Krueger & Casida 1957). Phosdrin was the highest and NC-13 the lowest in fly toxicity.

Systemic bioassays were made with 4- to 6-inch tall Perfection variety pea plants and fourth instar pea aphids, *Macrosiphum pisi* (Harr.). Systemic LD_{50} values were obtained with 10 aphids on each of two replicates with a dilution factor of two. Mortality readings were taken 24 hours after the aphids were placed on the plants. Phosdrin showed considerably higher systemic toxicity than OS-1808 and OS-1836 and over 100 times the systemic action of NC-13.

Properties of Some Components of Certain Substituted-Vinyl Phosphates.—Phosdrin, OS-1808, OS-1836 and DDVP were fractionated on silica gel columns prepared by mixing 100 ml. water with 140 grams silicic acid (Mallinckrodt chromatographic grade), slurring the gel in the organic solvent and packing into the column. In preliminary studies, two fractions were recovered from Phosdrin, two from OS-1808 and NC-13, three from OS-1836 and one fraction was recovered from DDVP. The solvents used to elute these fractions and the per cent composition of the technical materials are shown in table 2. The two fractions from NC-13 were found to be identical in all respects to the two fractions from OS-1808.

Elemental analysis of the alpha fraction of Phosdrin as eluted with carbon tetrachloride showed 4.9% chlorine. After stripping this alpha sample for 14 hours at room temperture at less than 0.5 mm., the chlorine content was reduced to 1.9%. The beta-chloroform fraction contained no chlorine. Elemental analysis on the stripped-alpha fraction of Phosdrin gave 37.0% carbon, 5.4% hydrogen, 13.5% phosphorus and 1.9% chlorine, and the beta fraction gave 37.6% carbon, 6.0% hydrogen, 13.7% phosphorus and 0.0% chlorine. Theory for Phosdrin analysis requires 37.5% carbon, 5.8% hydrogen, 13.8% phosphorus and 0.0% chlorine. The alpha and beta fractions therefore agree well with theory for the structure of Phosdrin. Partial infra-red spectra on these fractions are given in figure 1.

The stability of the various substituted-vinyl phosphate components was studied at pH 11 in an 0.1 *M.* borate-barbital buffer at 28° C. The degree of hydrolysis was determined by partitioning the aqueous reaction mixtures with an equal volume of chloroform and analyzing the total phosphorus content (method of Allen 1940) of each phase. The original organophosphate was recovered in the chloroform and the hydrolysis products in the aqueous phase. DDVP was less stable at pH 11 than any of the components of the other substituted-vinyl phosphates and the OS-1836 components were the most stable. The alpha fractions were less polar, less stable in alkali and more readily reduced permanganate than the beta fractions (table 2).

Anticholinesterase activity was determined with 30% whole human blood by a described method (Casida *et al.* 1956). The dimethyl phosphoryl components from Phosdrin yielded a very shallow slope for the anticholinesterase curves compared with the diethyl phosphoryl components of OS-1808, NC-13 and OS1836, indicating that the dimethyl phosphates may be combining with the cholinesterase in a slightly different manner. Other possible ex-

Table 2.—Properties of components from certain substituted-vinyl phosphates.

Property	Phosdrin		OS-1808[a]		OS-1836			DDVP One Fraction
	Alpha	Beta	Alpha	Beta	Alpha	Beta	Gamma	
Composition (%)	67	33	67	33	62	28	10	100
Chromatographic System (silica gel)	CCl_4	$CHCl_3$	*n*-hexane	*n*-hexane	CCl_4	CCL_4	$CHCl_3$	*n*-hexane
Partition coefficients								
CCl_4/H_2O	4.2	0.83	58	14	48	2.4	0.09	5.1
n-hexane/H_2O	0.32	0.059	5.5	1.1	6.5	0.30	0.03	1.7
Chemical reactivity								
Hydrolytic half-life (pH 11) (hours)	1.8	3.0	3.4	8.9	6.0	11.0	—	0.2
Oxidative half-life (MnO_4^-) (min.)	7.8	13.5	8.9	15.4	1.8	5.3	—	—
Biological activity								
pI_{50} blood ChE.[b]	6.1	4.4	7.8	5.9	5.9	4.9	—	3.7
LD_{50} (Subcut.) female white rat (mg./kg.)	0.25	25	0.35	35	12	60	400	6
LD_{50} (topical) female house fly (mg./kg.)	0.27	23	0.87	80	1	80	350	0.6

[a] Properties of NC-13 fractions were identical with those of OS-1808 but the ratio of the components was 5–9% alpha to 91–95% beta (Casida 1955).
[b] Negative logarithm of molar concentration of inhibitor resulting in 50% whole blood cholinesterase inhibition.

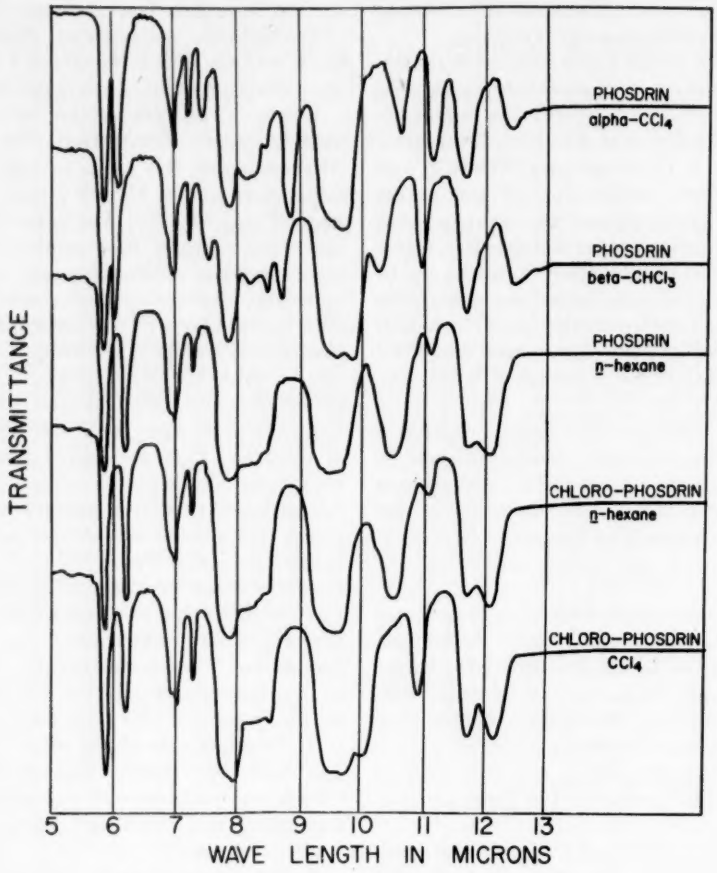


FIG. 1.—Infra-red spectra of Phosdrin components.

planations for the slope difference might be a greater hydrolysis rate or more rapid recovery of the inhibited cholinesterase with the dimethyl than with the diethyl phosphates studied. The alpha components were much more potent anticholinesterase agents than the beta components of all the compounds tested.

Acute mammalian toxicity was determined by subcutaneous administration of corn oil-acetone solutions with adult female white rats (150–200 grams) and the results serve only as an approximation (within a factor of two) of the LD_{50} value. The alpha components were also much more toxic than the beta components to house flies and white rats (table 2).

Presence of O,O-Dimethyl 1-Carbomethoxy-1-chloro-1-propen-2-yl Phosphate (Chloro-Phosdrin) as an Impurity in Technical Phosdrin.—Chromatography of technical Phosdrin on a silica gel column yielded one small fraction which eluted with *n*-hexane and two larger fractions which eluted with carbon tetrachloride and chloroform (table 3). The impurity present at 1.2 to 1.5% in technical Phosdrin was compared with known *O,O*-dimethyl 1-carbomethoxy-1-chloro-1-propen-2-yl phosphate (chloro-Phosdrin) (table 3 and fig. 1). When chloro-Phosdrin was chromatographed in an identical manner to Phosdrin, the major component was eluted with *n*-hexane and a second small fraction eluted with carbon tetrachloride in the position of alpha-Phosdrin. Based on infra-red spectra and partition coefficient studies, the impurity in technica Phosdrin appears to be alpha-chloro-Phosdrin. The beta fraction of chloro-Phosdrin chromatographed along with alpha-Phosdrin and may have contributed to the slight discrepancy in elemental analyses for this fraction. Chloro-Phosdrin undoubtedly originates from small amounts of 2,2-dichloracetoacetate formed in the chlorination of methyl acetoacetate to prepare the intermediate for synthesis of technical Phosdrin.

Chloro-Phosdrin was found to be about half as toxic as Phosdrin to rats (subcutaneous) and pea aphids (topical) and less than half as toxic to flies (topical).

Table 3.—Presence of *O,O*-dimethyl 1-carbomethoxy-1-chloro-1-propen-2-yl phosphate (chloro-Phosdrin) as an impurity in technical Phosdrin.

	Technical Phosdrin Components			Chloro-Phosdrin[a] Components	
	Alpha-CCl_4	Beta-$CHCl_3$	*n*-Hexane	Alpha-*n*-Hexane	Beta-CCl_4
Per cent composition	66	33	1.2–1.5	95	5
Partition coefficient					
CCl_4/H_2O	4.22	0.83	10.8	10.8	5.8
n-hexane/H_2O	0.32	0.059	0.88	0.95	0.59

[a] *O,O*-dimethyl 1-carbomethoxy-1-chloro-1-propen-2-yl phosphate prepared by reacting trimethyl phosphite and methyl 2, 2-dichloroacetoacetate. This material was provided by the Shell Development Company, Modesto, California.

Isomerization of Phosdrin and OS-1808 Components with Ultra-Violet Light.—Purified alpha and beta fractions from Phosdrin and OS-1808 were irradiated in chloroform solutions at 60° C. with strong ultra-violet light from mercury vapor or cadmium lamps. Each of the alpha and beta Phosdrin fractions shifted to partitioning properties intermediate between the alpha and beta materials. Irradiation of either the alpha or beta OS-1808 fractions yielded a mixture of the components. A progressive shift in solubility properties and infra-red absorption characteristics occurred with samples taken at successive time intervals during irradiation. After 8 hours of irradiation the products from the alpha and beta components of OS-1808 yielded identical infra-red spectra due to a mixture of about 70% beta and 30% alpha fractions.

Toxicity to House Flies of Mixed Alpha and Beta Fractions of OS-1808.—The alpha and beta fractions of OS-1808 were mixed in various proportions and the toxicity of the mixtures to house flies determined. The toxicity of the alpha fraction was not affected by the beta component in ratios as high as 10 parts beta to one part alpha (table 4).

Table 4.—Toxicity to house flies of mixed alpha and beta fractions of *O,O*-diethyl 1-carbethoxy-1-propen-2-yl phosphate (OS-1808).

PER CENT ALPHA IN MIXTURE[a]	LD_{50} OF MIXTURE AS μG./GM.[b]	μG. ALPHA/GM. FLY AT LD_{50} DOSAGE
0	70	—
5	13	0.65
10	8.7	0.87
20	4.0	0.80
40	2.3	0.92
70	1.7	1.19
100	0.87	0.87

[a] Isomers purified by column chromatography.
[b] Based on mortality 5 hours after application of organophosphate in 1.3 microliters of acetone to the pronotum of 2-day-old female flies.

Effect of Amino Acids and Some Related Chemicals on the Hydrolyis of Alpha-Phosdrin.—The effect on the hydrolysis of alpha-Phosdrin was determined with 55 amino acids and related chemicals at 0.1 *M.* and pH 9.0 (table 5). The best catalysts were found to be dl- and l-proline, 1-hydroxyproline, hydroxylamine hydrochloride, catechol, 3,4-dihydroxyphenylalanine and pyridine-aldoxime methiodides and dodeciodides. When certain of these compounds were tested as catalysts at 0.1, 0.01, and 0.001 *M.* and pH 9.0, the best activity resulted from pyridine-2-aldoxime methiodide and 1-hydroxyproline (table 6). Further study with 0.01 *M.* catalyst and pH 7.5 and 9.0 showed the pyridine-aldoxime methiodides to be superior to 1-hydroxyproline (table 7).

The effect of glycine on hydrolysis of organophosphates was further studied. Alpha-Phosdrin-P^{32} was incubated with 0.1 *M.* glycine at pH 7.5 for 24 hours and the nature of the hydrolysis products determined with an ion-exchange resin (Plapp & Casida 1958). Only dimethyl phosphoric acid was formed on hydrolysis under these conditions. The increase in hydrolysis rate in 0.1 *M.* glycine at pH 9.0 over that in 0.1 *M.* borate-barbital buffer at pH 9.0 was found to be 48 times for alpha-Phosdrin, 33 times for DDVP, 6 to 7 times for malathion and para-oxon and 2 times for *O,O*-diethyl S-(2-diethylaminoethyl) phosphorothiolate.

Table 5.—Effect of some amino acids and related chemicals at 0.1 *M.* and pH 9.0 on the hydrolysis of alpha-Phosdrin.

PER CENT HYDROLYSIS	COMPOUNDS
27–34—Not significantly higher than borate-barbital buffer alone[a]	d-alanine, l-alanine, dl-homoserine, glutamic acid, tyrosine, imidazole, phenylglycine, l-phenylalanine, β-phenylalanine, tryptophane, dl-citrulline, creatinine, betaine hydrochloride, creatine, hippuric acid, *p*-aminobenzoic acid, 2,5-dinitrophenol, dl-aminobutyric acid, β-amino isobutyric acid, tris-(hydroxymethylaminomethane), 4,5,6 hydroxy-2,7 naphthalene sulfonic acid, cobalt chloride, cadmium sulfate, sodium borate, chymotrypsin[b]
35–55	l-isoleucine, d-leucine, dl-norleucine, l-leucine, l-valine, β-alanine, l-threonine, d-serine, l-serine, aspartic acid, dimethylglyoxime, *p*-hydroxybenzoic acid, *p*-aminoethanoic acid
56–80	glycine, dl-allothreonine, l-histidine, dl-histidine, ammonium molybdate, dimethylamine hydrochloride
81–95	dl-proline, l-proline, hydroxylamine hydrochloride, catechol
96–99	3,4-dihydroxyphenylalanine, l-hydroxyproline, pyridine-2-aldoxime methiodide, pyridine-3-aldoxime methiodide, pyridine-4-aldoxime methiodide, pyridine-2-aldoxime dodeciodide.

[a] Boric acid and sodium barbital each at 0.05 *M.* and pH 9.0 . All other compounds were added to this buffer at 0.1 *M.* and the buffer readjusted to pH 9.0. before adding the alpha-Phosdrin at 500 p.p.m. After 24 hours incubation at 28° C. the pH was rechecked and each mixture was extracted with an equal volume of chloroform and the total phosphorus content of the aqueous phase was considered to be hydrolysis products.
[b] Crystalline chymotrypsin at 1 and 10 mg./ml. at pH 6.5 and 8.5, in the presence of 0.1 *M.* glycine, gave no more Phosdrin hydrolysis than did glycine alone.

Relation of Structure to Hydrolytic Stability and Biological Activity of Certain Substituted-Vinyl Phosphates.—The substituted-vinyl phosphates indicated in table 8 were prepared by reaction of trimethyl or triethyl phosphite with alpha-chloro aldehydes, ketones, or carboxylic acid esters according to the method of Allen & Johnson (1955). All products were purified on silica gel columns and the per cent of the alpha fraction based on total phosphorus was determined. Compounds 17 to 22 inclusive, in table 8 exist in only one isomeric form.

Infra-red spectra on the alpha and beta fractions from the various derivatives showed certain consistent shifts in absorption peaks which may be associated with the isomeric configuration. With the compounds listed in table 8 where R_2 is hydrogen or methyl, R_3 is hydrogen or

Table 6.—Effect of varying concentrations of certain amino acids and related compounds on the hydrolysis of alpha-Phosdrin.[a]

COMPOUND	PER CENT HYDROLYSIS AT CONCENTRATIONS INDICATED[b]	
	0.1 *M.*	0.01 *M.*
Pyridine-2-aldoxime methiodide	97	76
1-hydroxyproline	97	48
Hydroxylamine hydrochloride	97	47
l-proline	94	44
3,4-dihydroxyphenylalanine	88	42
Catechol	57	34
Glycine	57	27
Borate-barbital buffer	28	22

[a] Conditions other than those stated were identical to those used in table 5.
[b] The hydrolysis of Phosdrin with these compounds at 0.001 *M* was the same as with borate-barbital buffer alone.

Table 7.—Effect of pH on the hydrolysis of alpha-Phosdrin in the presence of 1-hydroxyproline and certain pyridine-aldoxime methiodides at 0.01 M.[a]

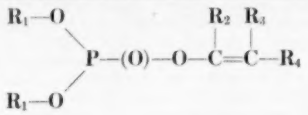
COMPOUND	pH 7.5	pH 9.0
Pyridine-2-aldoxime methiodide	83	98
Pyridine-4-aldoxime methiodide	77	95
Pyridine-2-aldoxime dodeciodide	67	84
Pyridine-3-aldoxime methiodide	53	95
1-hydroxyproline	13	65
Borate-barbital buffer	6	27

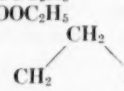
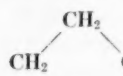
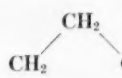
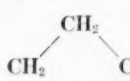
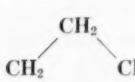
[a] Conditions other than those stated were identical to those used in table 5. The pyridine-aldoxime methiodides and dodeciodides were provided by Dr. I. B. Wilson, Columbia University, New York.

methyl and R_4 is chlorine or carbalkoxy (compounds 1 to 7, and 23), there is an absorption peak at 8.81 to 8.86 microns with the alpha fractions which is absent in the beta fractions. Absorption peaks were also present at 8.80 microns in compound 22, at 8.78 to 8.80 microns in compounds 17 to 21 and at 8.82 microns in the alpha fractions of compounds 13 to 16. An absorption peak at 11.00–11.10 microns was present in the alpha but not the beta fractions where R_2 is methyl or hydrogen. R_3 is hydrogen or chlorine and R_4 is chlorine or carbalkoxy (compounds 1 to 6, 23, and chloro-Phosdrin shown in figure 1, with the exception of compounds 3 and 22). A similar absorption peak appeared at 11.10–11.30 with the cyclopentenes (compounds 17 to 21), and at 11.10–11.25 in the alpha carbethoxy compounds (numbers 13 to 16). The peaks associated with the alpha isomeric configuration appear to be at 8.81 to 8.86 microns and at 11.00 to 11.25 microns.

The rate of alkaline hydrolysis was determined with the alpha fractions of these organophosphates at 500 p.p.m. in alkaline 0.1 *M.* borate-barbital buffer at varying hydroxyl ion concentrations using chloroform to water

Table 8.—Relation of structure to hydrolytic stability and biological activity of certain substituted-vinyl phosphates.[a]



COMPOUND NO.	R_1	R_2	R_3	R_4	PER CENT OF ALPHA IN TECHNICAL	APPARENT[b] K(OH⁻) (MIN.⁻¹)	ANTI-CHE. pI_{50}[c]	LD_{50} (MG./KG.) House Fly Topical-Female	LD_{50} (MG./KG.) White Rat (Subc.) Female
1	CH_3[d]	CH_3	H	$COOCH_3$	67	30	7.5	0.27	0.25
2	C_2H_5[e]	CH_3	H	$COOC_2H_5$	67	2	7.0	0.87	0.35
3	C_2H_5	CH_3	H	$COOC_3H_7$-*n*	81	8	7.0	3	1
4	C_2H_5	CH_3	H	$COOC_4H_9$-*n*	80	5	8.7	1	25
5	C_2H_5	CH_3	H	$COOC_4H_9$-*i*	86	5	7.0	1	35
6	C_2H_5	CH_3	H	$COOC_4H_9$-*s*	86	2	7.3	0.8	25
7	C_2H_5	CH_3	CH_3	$COOC_2H_5$	89	2	5.6	6	—
8	C_2H_5	CH_3	*i*-C_5H_{11}	$COOC_2H_5$	64	2	6.5	8	75
9	C_2H_5	C_6H_5	H	$COOC_2H_5$	88	4	8.0	1	3
10	C_2H_5	C_6H_5	C_2H_5	$COOC_2H_5$	86	2	7.1	25	3
11	C_2H_5	$CH_2COOC_2H_5$	H	$COOC_2H_5$	90	1150	6.8	38	13
12	CH_3	$COOC_2H_5$	H	$COOC_2H_5$	78	2300	7.1	50	50
13	C_2H_5	$COOC_2H_5$	H	$COOC_2H_5$	83	1400	8.0	6	1
14	C_2H_5	$COOC_2H_5$	CH_3	$COOC_2H_5$	71	55	7.5	8	3
15	C_2H_5	$COOC_2H_5$	C_2H_5	$COOC_2H_5$	74	60	8.0	13	3
16	C_2H_5	$COOC_2H_5$	*n*-C_3H_7	$COOC_2H_5$	74	14	5.6	10	6
17	C_2H_5			$COOCH_3$	94	9	5.1	8	25
18	C_2H_5			$COOC_2H_5$	92	4	5.0	12	200
19	C_2H_5			$COOC_3H_7$-*n*	94	2	4.7	75	300
20	C_2H_5			$COOC_3H_7$-*i*	92	0.5	6.9	25	250
21	C_2H_5			$COOC_4H_9$-*n*	96	3	5.0	38	300
22	CH_3[f]	H	Cl	Cl	100	60	6.0	0.6	6
23	C_2H_5[g]	H	H	Cl	62	1.3	5.4	1	12

[a] Alpha fractions from silica gel columns.
[b] Results for the first order hydrolysis reactions are expressed as "apparent" $K(OH^-)$ since not only the hydroxide ion but also the buffer constituents may have served as nucleophilic agents.
[c] Colorimetric method for activity of anticholinesterase agents (Shell Development Company 1956) using purified acetylcholinesterase obtained from Winthrop Stearns Inc.
[d] Phosdrin. [e] OS-1808. [f] DDVP. [g] OS -1836.

partitioning according to the method of Heath (1956) with total phosphorus determined colorimetrically on the separated phases. The least stable compounds were those in which R_2 contained a carbethoxy group (compounds 11 to 16). The dimethyl phosphates DDVP and alpha-Phosdrin (numbers 1 and 22) were also quite unstable. The stability was similar for several compounds (numbers 2 to 10 and 17 to 21) where R_2 is alkyl or aryl and R_3 is hydrogen or alkyl, or with the cyclopentene group including the double bond.

There did not appear to be a distinct correlation between stability, toxicity, and anticholinesterase activity. All the substituted-vinyl phosphates studied were very potent anticholinesterase agents. Except for the cyclopentene derivatives (numbers 17 to 21) the compounds were all highly toxic to flies and rats. The carbobutyryloxy derivatives (compounds 4 to 6) related to OS-1808 (compound 2) were the most selectively toxic to house flies compared with rats. The cyclopentene compounds (numbers 17 to 21) and the two chloro-vinyls (numbers 22 and 23) also showed some selectivity. Substitution of alkyl or cyclopentene groupings for R_3 reduced the anticholinesterase activity and toxicity to rats and flies over that with a hydrogen substituent in position R_3.

DISCUSSION AND SUMMARY.—The geometric isomers of substituted-vinyl phosphates can usually be separated by partition chromatography with silica gel columns. The isomer first eluted (alpha) is always less stable than the second isomer eluted (beta). The alpha is the more toxic isomer to insects and rats, the more potent antiesterase agent, the more rapidly attacked by permanganate and the less stable isomer in alkali and in plants (Casida *et al.* 1956). On irradiation with ultra-violet light the beta isomer predominates at equilibrium. Synthesis through the reaction of trialkyl phosphite with an alpha-chloro aldehyde, ketone or carboxylic acid ester yields predominantly the alpha isomer, while the reaction of dialkyl chlorophosphate with a salt of the carboxylic acid ester enol forms mostly beta isomer (Casida 1955). The first method of synthesis may yield chloro-vinyl phosphate impurities since *O,O*-dimethyl 1-carbomethoxy-1-chloro-1-propen-2-yl phosphate was found to be an impurity in technical Phosdrin. The presence of the beta isomer of *O,O*-diethyl 1-carbethoxy-1-propen-2-yl phosphate neither enchanced nor antagonized the toxicity of the alpha isomer to house flies. Infra-red spectra on the purified isomers of several substituted-vinyl phosphates showed that absorption peaks at 8.81 to 8.86 and 11.00 to 11.10 microns may be associated with the alpha isomeric configuration.

The isomeric configuration of the alpha and beta fractions from these substituted-vinyl phosphates is as yet uncertain. Deductions from synthesis routes and reactivity indicate that the alpha fractions probably have the dialkyl phosphate and chlorine or carbalkoxy groups cisoid to each other and the beta fractions have these groupings in transoid positions.

Of 55 amino acids and related chemicals studied for efficiency in catalyzing the hydrolysis of alpha-Phosdrin, the most active were hydroxyproline and certain pyridine aldoxime methiodides. Hydroxyproline has an imino nitrogen rather than an amino nitrogen as occurs in most of the other amino acids. Pyridine-2-aldoxime methiodide serves as an antidote for poisoning of mammals with some alkyl phosphates under certain conditions (Wilson *et al.* 1955, Loomis 1956, Wagner-Jauregg 1956, Askew 1957, Erdmann & Schmidt 1957, Kewitz 1957, Wills *et al.* 1957, Wilson & Sondheimer 1957, Brown *et al.* 1957).

The alpha fractions from 24 substituted-vinyl phosphates were compared as to alkaline stability, anticholinesterase activity and toxicity. No distinct correlation between stability, toxicity to rats and flies, and anticholinesterase activity was apparent.

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Insectary Technique for Testing the Resistance of Small Grains to the Greenbug[1]

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ABSTRACT

A technique is described for testing the resistance of small grains to the greenbug (*Toxoptera graminum* (Rond.)) in a controlled-environment insectary having a year-round average temperature of 75° F. and in which artificial light is used. Test varieties grown in flats covered with plastic cages are subjected to uniform infestation for 10 to 14 days, and then rated according to the leaf area damaged on each plant. Since the environment is well controlled, differences in reaction of the plants to the greenbugs are most likely due to their inherent characteristics.

The greenbug (*Toxoptera graminum* (Rond.)) is a pest of small grains in the Central and Southwestern States, where it causes serious damage in outbreak years. Control measures have been developed, but they are not always practical. The use of insecticides is limited by cost where grain yields are low due to climatic or other conditions, or by their ineffectiveness at the low temperatures at which greenbugs normally reproduce. Parasites, predators, and cultural control methods are frequently not effective. Consequently, more satisfactory controls are needed. Resistant varieties of small grains offer a promising approach to practical control.

The literature on greenbug resistance in small grains has been reviewed by Dahms *et al.* (1955). They and others have recorded differences in resistance among wheat, oat, and barley varieties. The differential reaction of Wintex and Omugi barleys to greenbug infestation under insectary conditions is shown in figure 1. However, adapted greenbug-resistant varieties of these crops are not yet available to farmers. Resistant germ plasm is needed for use in a breeding program.

In 1947 Dahms (1955) developed a technique for testing the resistance of small gains to greenbug attack. It involved growing test varieties in pots in a greenhouse, subjecting the plants to infestation by greenbugs of uniform age, and recording data on plant tolerance, greenbug preference, and greenbug fecundity. Through use of this technique germ plasm of definitely known resistance was made available to plant breeders for the first time.

This technique is satisfactory for examining a limited number of varitieies whose performance or parentage indicates possession of resistant characteristics. In searches for resistant germ plasm among large numbers of varieties, such as the world collections of small grains where little is known regarding their reaction, this technique involves too much time, and more data are obtained than are necessary on varieties that show no resistance. The gross separation of these varieties into resistant and susceptible categories would suffice for this purpose. An insectary technique for screening small grains for greenbug-resistant germ plasm in a rapid manner developed at Denton, Texas, is described herein.

MATERIALS AND METHODS OF TESTING.—The testing is done in a controlled-environment insectary (fig. 2), 24×12 feet in size. The walls and ceiling are insulated. An average temperature of 75° F. is maintained by means of two ¾-ton refrigerated air conditioners and an electric heater, each thermostatically controlled. Light is supplied for 16 hours each day—6 A.M. to 10 P.M.—by fluorescent fixtures, each consisting of four 40-watt tubes suspended 21 inches above a table top, or several inches above the tops of cages. Two of the tubes in each fixture are the daylight and the other two are the cool white type. The lights are controlled by a brooder-house clock. Relative humidity is not controlled, since it remains between 50 and 60%, which is satisfactory.

Cypress flats are used for growing the test varieties. Inside dimensions are $22\frac{1}{2}\times12\frac{1}{2}\times3\frac{1}{2}$ inches. Each flat accommodates 14 rows of 10 plants—12 rows of test varieties and one resistant and one susceptible check.

A cage that just fits inside the flat is used to cover the test plants and confine the greenbugs (fig. 2). The sides and top are of cellulose nitrate plastic (Pyralin). Acetone is used to seal the plastic joints. Wood strips $\frac{3}{4}\times\frac{1}{2}$ inch are used for reinforcement. The plastic is fastened to the wood strips by means of a hand-operated stapletacker. The bottom reinforcing strips are fastened inside one-half inch from the bottom edge. In the top of the cage is an opening $12\times4\frac{1}{2}$ inches for ventilation, and it is

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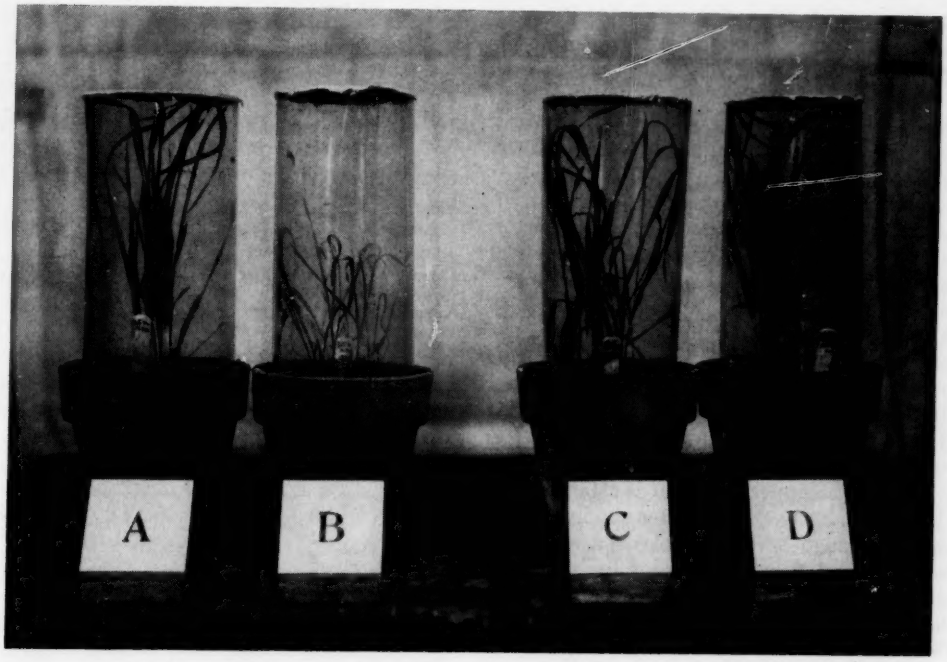

FIG. 1.—The reaction of Wintex (A and B) and Omugi (C and D) barleys to the greenbug after being infested for 2 weeks. B and D infested plants.

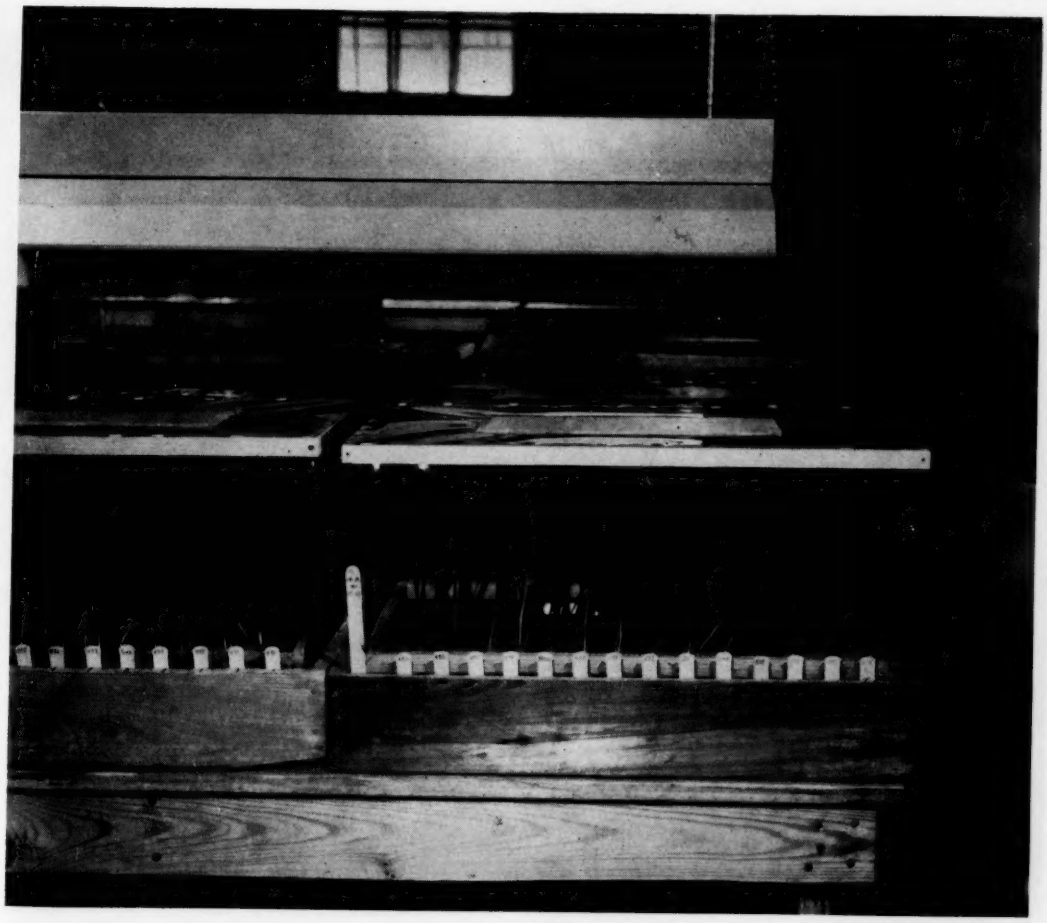

FIG. 2.—General view of the insectary, showing flats, cages, and lights.

covered with 34-mesh plastic screen attached with acetone.

Kirvin fine sandy loam from the East Crosstimbers region near Denton is used in the flats. Heavy soils such as the clays should be avoided, because they stick and harden after being wet and it is difficult to remove the cage without damaging the plants.

Uniform greenbug cultures are maintained in the insectary on plants grown in pots. Susceptible varieties such as Wintex barley, Mustang oats, and Pawnee wheat are used as culture plants in order to promote rapid greenbug development. When the plants are 10 days old, they are infested and covered with a cylindrical plastic cage. After another 10 days the greenbugs are used for infesting plants in the flats.

The soil in the flats is soaked at planting and again 1 week after the plants have been infested. Watering after infestation is accomplished by lifting one side of the cage slightly and inserting a copper tube that is attached to a small hose leading to an overhead water supply. Water flows by gravity through 1/16-inch holes spaced 1 inch apart in the tube, and flow is regulated by a sprayer valve. Pressure directly from a hydrant is too great and will wash the soil away from the plants.

To ensure a uniform stand and avoid the necessity of thinning, the seeds are germinated between wet paper toweling. After 3 days in a germinator, viable seeds have developed primary roots and a stem shoot. Ten seedlings of each test variety and of the resistant and susceptible checks are then planted in rows across the flat (fig. 3), five of each variety in consecutive order in one-half of the flat, and the remaining five randomized in rows in the other half. The resistant check is placed in the fifth row and the susceptible check in the tenth row. A wooden stake bears the entry number of the variety in each row. The seedlings are then covered with one-half inch of sand and watered. The cage is placed over the flat, and the bottom edge is pressed into the soil to keep greenbugs that might be free in the insectary from infesting the developing plants.

Growth is rapid under the insectary conditions, and in 1 week the plants are 3 to 4 inches tall. They are then infested with greenbugs at the rate of five per plant.

Each plant is examined for damage, and a tolerance rating is made when some of the plants in the flat, usually in the susceptible check, have been killed by greenbug feeding. This is about 14 days for wheat, and 10 days for oats and barley. The ratings, based upon the area damated, are as follows:

Tolerance Rating	*Per Cent of Leaf Area Damaged*
0	0–10
1	11–20
2	21–40
3	41–60
4	61–80
5	Beyond recovery

The average rating of each variety is also expressed as a percentage of the resistant check in order to overcome slight variations sometimes occurring between flats. Only varieties having tolerance ratings lower than the resistant check are saved for further testing. In the insectary space available 144 varieties per week are tested in this manner.

The resistant varieties are subjected to a plant-longev-

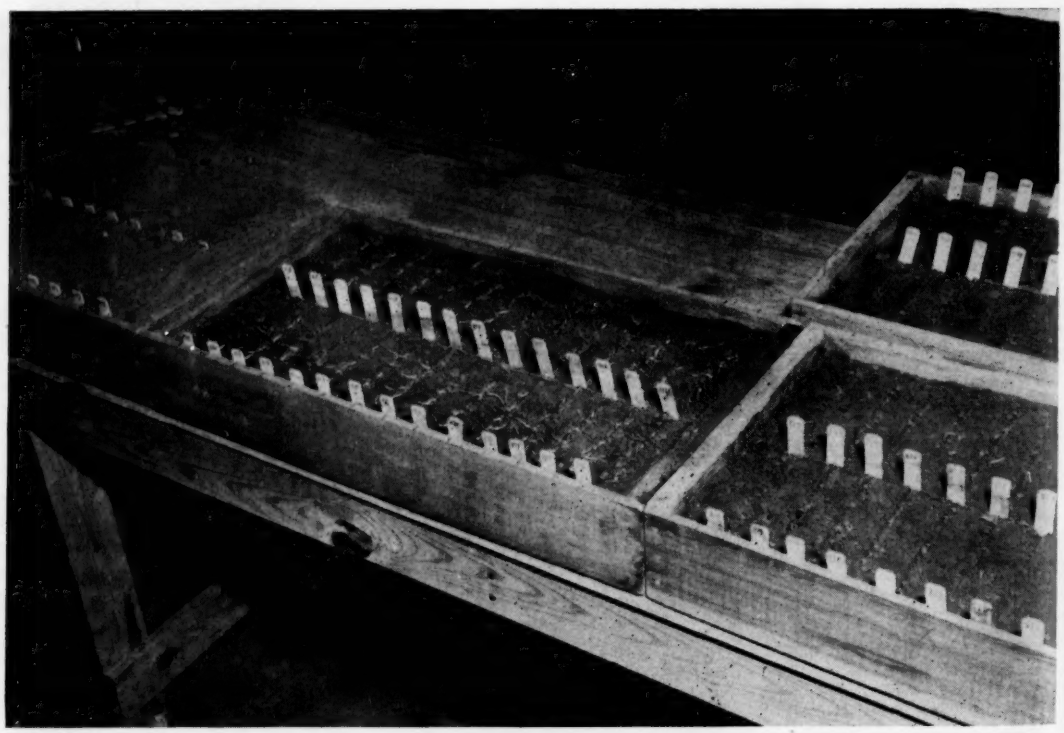

FIG. 3.—Grain seedlings in flats before being covered with soil.

ity tolerance test, using the same resistant and susceptible checks. Twenty plants of each variety and 10 each of the checks are planted in the test flats. The procedure followed is the same as described above, except that tolerance ratings are not made and the greenbugs are allowed to develop until the plants are killed. The number of days the plants live is recorded, and longevity of the variety is expressed as a percentage of the longevity of the resistant check. This is a severe test for the surviving plants. They are subjected to heavy infestations by greenbugs leaving the susceptible plants that have been killed.

Frequently individual plants of a variety are resistant and remain alive after the other plants have been killed. After all greenbugs have been removed, these plants are transferred to pots and placed in a greenhouse, where they are kept until they produce seed. These segregates, or possibly mixtures, in a variety may be the source of resistant germ plasm.

All the varieties subjected to the longevity tolerance test are also tested for antibiosis, in which data are obtained on the progeny of a single female on an individual plant. Six plants of each variety are planted in two 6-inch pots. One week after germination a single female that has not reproduced is placed on each plant which is then covered with a plastic cage $10 \times 2\frac{3}{8}$ inches. Seven days later counts are made of the progeny of each greenbug.

The reaction of hybrids with greenbug-resistant parents is also observed in the insectary. Nursery selections of each hybrid in each generation are tested for reaction to greenbugs, and those more susceptible than the resistant parent are eliminated from further planting. It has been found that the percentage of resistant selections of the various crosses has been increased from year to year.

DISCUSSION.—All variable factors that might affect greenbug development and plant growth are controlled fairly well in this insectary. Therefore, any differences in reaction of the plants to greenbug infestation are most likely due to their inherent characteristics. Since a uniform procedure is used, results are more consistent than those obtained under variable conditions that exist in a greenhouse. Furthermore, it is possible to conduct tests throughout the year, whereas under Texas conditions high temperatures limit greenhouse testing to several of the winter months.

Although this insectary technique has been devised for greenbug resistance studies, it could easily be adapted for studies on other insects.

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Experiments with Protein Hydrolysate Bait Sprays for Control of the Walnut Husk Fly[1]

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ABSTRACT

A controlled corn-protein hydrolysate, Staley's Protein Insecticide Bait No. 7, was shown to be the most attractive of the materials tried as attractive components of toxic bait sprays for experimental use in control of the walnut husk fly (*Rhagoletis completa* Cress.). Malathion wettable powder proved superior to technical Dipterex as a toxic component. Bait sprays were compared with standard full-coverage sprays of malathion (without attractant) in area control of adults (a) by observing their effects on the fly population as indexed by bait pails containing glycine-sodium hydroxide solution and (b) by recording larval infestation of walnuts. Comparison of treatments, on the basis of the length of time that fly populations were controlled, showed that bait-type spray coverage without the protein hydrolysate attractant strongly depressed fly populations for 5 to 7 days, and with the attractant, for 12 days. Standard full-coverage sprays (using twice the amount of malathion) depressed fly populations for 9 to 11 days. Very good control of the walnut husk fly resulted from two bait sprays or from standard full-coverage treatments spaced at a 25-day interval. Although fly populations built up during the second half of this interval, little oviposition occurred. However, bait sprays were observed to result in high populations of *Tetranychus cinnabarinus* (Boisd.) on Placentia and Payne walnut varieties during the second year of trial. Such mite infestations did not occur in solid blocks of Eurekas, a variety very susceptible to walnut husk fly but less susceptible to this species of mite than the other varieties listed.

The use of protein hydrolysates in toxic bait sprays for fruit fly control has been developed by Steiner (1952, 1954, 1957). Studies on the potential use of protein hydrolysates in bait sprays for control of the walnut husk fly (*Rhagoletis completa* Cress.) were undertaken in 1955. The first objective was to ascertain whether protein hydrolysates are attractive to walnut husk flies. With this question answered, the studies led to the selection of the most attractive of these materials, to the selection of a satisfactory toxicant, and to extensive field experiments on area control of flies with bait sprays applied variously by aircraft, semi-concentrate air-blast, or standard high-pressure sprayers.

MATERIALS AND METHODS.—The foliage deposit and tray technique of Steiner (1952) was used in studies comparing various materials as bait-spray attractants. It is here noted that this technique is much preferred to others in studies seeking information on attraction of fruit flies

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to bait-spray deposits on foliage. Screening these materials as attractants in bait pans, sticky traps, etc., may give misleading results with reference to their utility in bait sprays. This is related to a number of factors, *e.g.*, to the decay of protein hydrolysates in water solution altering olfactory stimuli, and to the intimate bearing of the method of exposure of the attractant on its performance. In the tray technique used in these studies, measured amounts of a solution of protein hydrolysate and a rapidly acting toxicant are painted on both surfaces of a standard area of foliage, beneath which a screen tray (2′×2′) is suspended. Flies are attracted to and feed upon the deposit. They are rapidly affected by the poison and fall into the trays, where they are counted daily. Tray tests were replicated 4 to 10 times.

Effects of area treatments on control of adults in walnut orchards were measured in two ways: (1) by direct effects on the adult population and (2) by degree of protection afforded against infestation of walnuts. Husk fly populations were indexed in each orchard before and after treatments with bait pans containing glycine-sodium hydroxide bait solution. After first aging for 2 weeks, this bait solution has a desirable characteristic of relatively constant power of attraction for at least 7 weeks (Barnes & Ortega 1958). Five bait pans (1½-quart, gray-enameled pudding pans) were run in each orchard. Counts were made shortly before harvest, recording the percentage infested of all nuts that could be reached from the ground on five trees in each orchard if the infestation was obviously at a trace level (infested walnut hulls turn black) or on 10 trees if a significant infestation was present. Orchards of the Eureka variety were used for walnut husk fly control studies because, of the commercial varieties of Persian walnuts grown in southern California, this variety is the one most susceptible to attack. The entire planting in each case was treated or untreated, replication being by orchard. In studies of the effects of the treatments on populations of mites, observations on the Placentia and Payne walnut varieties were also made.

RESULTS.—*Feeding-Attractant Studies.*—For observation of the response of flies to foliage deposits of partially hydrolyzed yeast protein (PHYP), an acid hydrolysate, a preliminary tray trial was installed in a Simms peach orchard having a high population of walnut husk flies. The results (table 1) show that foliage deposits of PHYP have a very high degree of attractiveness for walnut husk flies.

Following the initial results of the trial reported in table 1, a tray experiment arranged in an 8×8 Latin square was installed in the same orchard (1) for comparing malathion and Dipterex as toxicants with PHYP, and (2) for comparing foliage baits of PHYP with those of (a) liquid corn protein hydrolysate (Staley's Protein Insecticide Bait No. 2), (b) an enzymatic hydrolysate of yeast (Protein Hydrolysate-MRT), and (c) glycine.

Results of this trial (table 2) indicate the superiority of a wettable powder of malathion to technical Dipterex in providing rapid mortality when used with PHYP. The higher concentration of Dipterex resulted in a lower catch, suggesting taste repellency. Of the three protein hydrolysate materials used, the corn protein hydrolysate was superior to the other two. This product contained 34% amino acid and amino acid salts, 14% sodium chloride, and 4% ammonium chloride. Over four times as many flies were recovered with the corn protein hydrolysate product as with PHYP, though the latter was used at a higher concentration of proteinaceous solids. Glycine deposits were weakly attractive. About 90% of the flies caught with each of the bait attractants under the conditions of this trial were females (see table 2).

A trial was then conducted to compare malathion and Dipterex as toxicants in combination with the corn protein hydrolysate. Results (table 3) again showed much lower catches of husk flies with Dipterex than with malathion; the higher concentration of Dipterex caught about half as many flies as the lower concentrations, again suggesting taste repellency. Malathion was therefore used as the toxicant in subsequent trials.

Since the liquid corn protein hydrolysate product (Staley No. 2) used contained a relatively high amount of salts, observations were made in 1955 on possible injury to foliage. None was observed in the peach foliage trials noted above, or following two applications of 1 quart per 100 gallons on Eureka walnuts.

Orchard Spraying Experiments, 1956.—The tray trials of 1955 had demonstrated that foliage deposits of corn protein hydrolysates are highly attractive to walnut husk flies and that flies would readily ingest a lethal dose of malathion when feeding on such deposits. In 1956, orchard trials were carried out to develop information on the possible use of bait sprays for commercial control of walnut husk fly. It is apparent that bait sprays are intended to provide area control of adults (a) by destroying the adult population present in an orchard at the time of

Table 1.—Preliminary tray trial with partially hydrolyzed yeast protein (PHYP) as feeding attractants to walnut husk flies in a Simms peach orchard.[a]

Treatment	(Weight—Per Cent in Water)	Number Dead Flies per Tray at Intervals After Treatment[b]									
		Hours			Days						
		1	2	3	1	4[c]	5	6	8[c]	11[c]	13[c]
PHYP[d]+Dipterex 50	5.8 4.3	18	16	7	197	65	78	139	97	19	7
Dipterex 50	4.3	5	0.8	0.6	2	2	1	1	1	8	5

[a] Trial instituted August 18, 1955, with five replicates.
[b] Flies removed from trays at each inspection.
[c] Total number of flies divided by number of days since preceding inspection to give average number of flies per day for given interval.
[d] M. R. Thompson Company, Stamford, Connecticut.

Table 2.—**Protein hydrolysates as attractants for walnut husk fly in combination with rapidly acting toxicants in deposits on peach foliage in 1955.**[a]

TREATMENT[b]	(WEIGHT—PER CENT IN WATER)	NO. OF FLIES CAUGHT PER TRAY								PER CENT FEMALES[d]
		Aug. 25[c]	Aug. 26	Aug. 27	Aug. 28	Aug. 29	Aug. 31	Sept. 1	Total	
Dipterex (tech.)	1.2	1.3	0.7	0.1	0	0.2	0.1	0	2.4	—
PHYP[e]+Dipterex (tech.)	2.4 1.2	25.5	8.4	3.0	0.7	0.9	0.7	1.5	40.7	85
PHYP+Dipterex (tech.)	2.4 0.6	38.3	16.1	3.0	1.6	1.5	1.5	1.8	63.8	89
PHYP+Malathion, 25% W.P.	2.4 4.8	55.5	18.0	3.1	1.0	1.5	3.5	0.4	83.0	91
Glycine+Dipterex (tech.)	2.4 1.2	10.2	2.3	1.1	0.1	0.5	0.3	0	14.5	97
MRT[e]+Dipterex (tech.)	2.4 1.2	46.7	28.5	6.4	2.7	2.8	4.7	3.0	94.8	89
Staley No. 2[e]+Dipterex (tech.)	2.4 1.2	75.0	28.7	6.2	4.0	1.5	1.5	0.5	117.4	90
Staley No. 2+Dipterex (tech.)	4.8 1.2	123.0	38.2	6.7	4.4	3.9	3.2	0.7	180.1	92

[a] Experiment arranged in an 8×8 Latin square in a tree block 16×16 within a 20-acre orchard, using every other tree.
[b] Ten milliliters of solution painted on both sides of leaves of seven peach terminals, which were tied over screen trays to catch flies as they fell. Treatments applied row by row in Latin-square sequence.
[c] Number of flies per tray after completing treatment, Aug. 24–25.
[d] In total catch of August 26.
[e] PHYP, partially hydrolyzed yeast protein, an acid hydrolysate; MRT, enzymatic hydrolysate of yeast protein; M. R. Thompson Company, Stamford, Connecticut. Staley No. 2: Protein Insecticide Bait No. 2 an acid hydrolysate of corn protein; A. E. Staley Mfg. Company, Decatur, Illinois.

application and (b) by holding the adult population at a low level by residual insecticide action for a certain period of time, despite continuing emergence.

After the residual effect of the bait spray is lost, additional protection is required at once if flies are moving into the orchard from the outside. If, however, the new population is derived almost entirely from emergence within the orchard, then after the residual effect of the bait spray is lost, additional protection is not required until the minimum pre-oviposition period elapses. The walnut husk fly has a pre-oviposition period of about 18 days (Boyce 1934). In the 1956 trials, the assumption was made that for commercial control re-treatment would not be required until about 2 weeks after the residual effect of the treatment was lost, as indicated by resurgence of catches in the bait pails.

Table 3.—**Comparison of Dipterex and malathion as walnut husk fly toxicants in combination with corn protein hydrolysate.**

SPRAY TREATMEMT	(WEIGHT—PER CENT IN WATERS[a])	TOTAL CATCH OF FLIES
Malathion 25% W.P.+Staley No. 2	4.8 2.4	172
Dipterex (tech.)+Staley No. 2	0.6 2.4	98
Dipterex (tech.)+Staley No. 2	1.2 2.4	55

[a] Peach foliage over tray painted with 10 ml. of toxicant. Four replicates, September 2 to 20, 1955.

The orchard spraying trials were organized for two purposes: (1) to obtain evidence as to whether a bulk spray program of two applications of 10 pounds of 25% malathion wettable powder in 500 gallons water per acre provides control of walnut husk fly, a program referred to as the "standard" program, consisting of standard spraying methods, but not yet sufficiently validated as affording standard control, and (2) to determine the effectiveness of bait-spray programs using corn protein hydrolysate products in combination with reduced dosages of malathion for control of walnut husk fly. Bait-spray programs were applied as described in table 4, by aircraft, by air-blast sprayer, and by hand guns.

Results of these trials in terms of infested nuts at harvest are presented in table 4. It should be recognized that the orchards in the Puente area and at Sunnymead had a light carryover of husk fly because of extremely high temperatures during the first 3 weeks of September, 1955, which reduced larval infestations. Orchards at Beaumont, Calimesa, and Yucaipa, however, had reasonably high infestations carrying over from 1955. Since entire orchards were used, results of one season may not be considered conclusive, variation in levels of infestation from orchard to orchard not being sufficiently controlled by the limited orchard-to-orchard replication. However, results in 1956 were consistent in the sense that a treated orchard in a given area invariably had a lower infestation than an untreated orchard, apparently demonstrating control resulting from the treatments. These results were appar-

Table 4.—Infestation of Eureka walnuts by walnut husk fly at harvest after described treatment schedule, 1956.

Treatment and Orchard No.	Location	Acres	Equipment	Material/ Acre	Gallons Water/ Acre	Treatment Dates	Per Cent Nuts Infested
A. Check							
1	Yucaipa	3		Untreated			22
2	Beaumont	5		Untreated			64
3	Sunnymead	4		Untreated			8
4	Puente	0.25		Untreated			22
B. Full Coverage							
5	Yucaipa	3	Speed Sprayer[a]	Malathion 25% W.P. 10 lbs.	500	8/7, 8/28	2
C. Bait Spray—Aircraft							
6	Beaumont	14	Aircraft	Malathion 25% W.P. 4 lbs. Staley No. 2, 2 qts.	10	8/10, 9/3	2
7	Sunnymead	10	Aircraft	As above	10	8/10, 9/3	0
D. Bait Spray							
8	Yucaipa	5	Speed Sprayer	As above	200	8/7, 8/28[b], 9/14	2
9	Puente	15	Hand guns	As above	200	8/13, 9/8	5
10	Puente	45	Hand guns	Malathion 25% W.P. 4 lbs. Staley No. 7, 2 qts.	200	7/30, 8/20	0.1
E. Bait-Type Spray (No Bait)							
11	Yucaipa	1	Speed Sprayer	Malathion 25% W.P. 4 lbs.	200	8/7, 8/28,[b] 9/14	6

[a] Model 40 with volute in all trials. Standard nozzles in all trials.
[b] Treatment on this date was at one-half the dosage and rate of the previous date. Control of adults was not adequate and a third treatment was required.

ently in the direction of excellent control by the full-coverage standard sprays of malathion and by bait sprays applied by aircraft, and of satisfactory control by bait sprays applied by speed sprayer or hand guns. No foliage injury was observed from any of the treatments.

Effects of aircraft-applied bait sprays on the husk fly population in an isolated orchard (orchard 6, table 4) are shown in figure 1, in comparison with the husk fly population in an untreated orchard approximately 5 miles distant. To explain the dramatic effect of the first treatment, it is possible that emergence may possibly have been complete in this isolated orchard when the first treatment was made (fig. 1).

A tray trial was carried out in 1956 to compare attractive value of two corn protein hydrolysate products with that of soy bean protein hydrolysate. The hydrolysates were used by weight in water at 5% plus 5% of a 25% malathion wettable powder. Results with five replicates were as follows:

Material[3]	*Total No. Flies 9 Days After Treatment*
Staley No. 7	210
Staley No. 2	130
Soy hydrolysate	48

The Staley No. 7 product, which is described as a controlled corn protein hydrolysate, was superior. It will be noted that a bait spray schedule in 1956 with the No. 7 product (orchard 10, table 4) provided excellent control.

Orchard Spraying Experiments, 1957.—Fifteen Eureka walnut orchards were used in 1957 for testing the effectiveness of bait sprays of malathion combined with corn protein hydrolysate, in comparison with that of malathion alone, and with that of standard full-coverage malathion sprays. Since concentrated aircraft-applied sprays had been very effective in 1956, the amount applied in ground equipment treatments was reduced from 200 to 100 gallons per acre. Since the controlled corn protein hydrolysate (Staley's Protein Insecticide Bait No. 7) had provided superior attraction in 1956, it was adopted for experimental use in orchard trials in 1957.

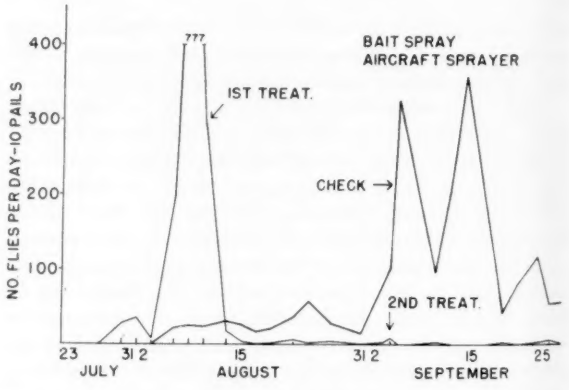

FIG. 1.—Effects of aircraft-applied bait sprays of malathion and corn protein hydrolysate (Staley No. 2), on walnut husk fly population in an isolated 14-acre Eureka walnut orchard (orchard 6, table 4), as indexed by glycine-sodium hydroxide bait pails installed July 25, and in comparison with walnut husk fly flight in an untreated orchard (see orchard 2, table 4) in 1956.

[3] Supplied by A. E. Staley Mfg. Company.

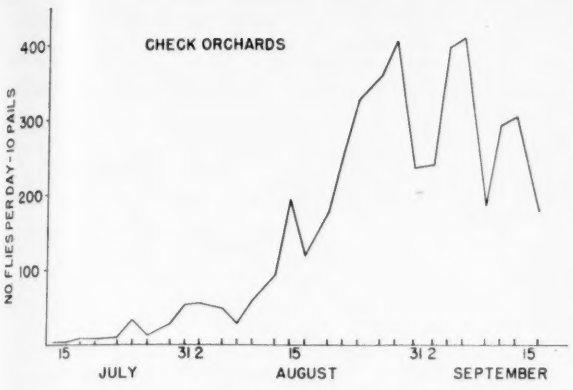

FIG. 2.—Average bait-pail catches per day of walnut husk flies in three untreated Eureka walnut orchards (1, 2, and 3; table 6) in 1957.

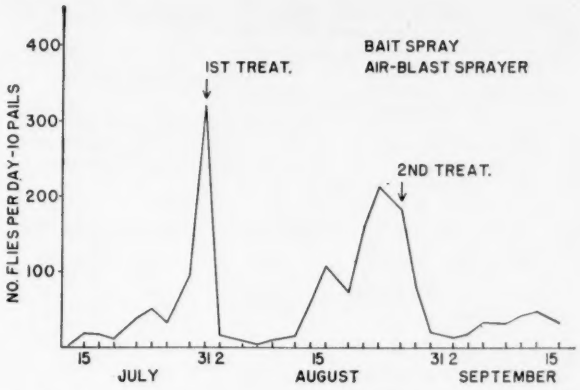

FIG. 4.—Average bait-pail catches per day of walnut husk flies in three Eureka walnut orchards (10, 11, and 12; table 6) in relation to time of treatment with bait sprays applied by air-blast sprayers in 1957.

As before, the effectiveness of treatments was studied by observing their effects on the walnut husk fly adult population by means of bait-pail returns in treated and untreated groves, and by counts at intervals through the season and at harvest of the percentage of stung fruits. The number of orchards included in each treatment was as follows: check (untreated), 3; full-coverage, 3; aircraft bait-spray, 3; air-blast sprayer bait-spray, 4; and bait-type spray without bait (hydrolysate), 2. Walnut husk fly catches in orchards of each category were averaged and are charted in figures 2–6, in which treatment dates are also shown.

Bait pails were installed in all orchards on July 1. Bait-pail catches indicated that emergence began in most orchards in mid-July. Figure 2 shows that in the check orchards the peak flight period in 1957 was from August 15 to September 15. The effects of the different treatments upon flight of adults are expressed in table 5 in terms of percentage reduction in flight for the 10 days after treatment, as compared with that of the 10 days before treatment in the same orchards. On this basis the treatments rank as follows: (1) bait sprays, (2) full-coverage sprays, (3) bait-type spray without bait (table 5). On the basis of resurgence of catches in bait pans, the initial bait-spray treatments apparently exerted control of adults for a period of 12 days (figs. 3 and 4). The initial full-coverage sprays depressed bait-pail catches from 9 to 11 days (fig. 5). Bait-type sprays without bait (protein hydrolysate) depressed catches for 5 to 7 days (fig. 6).

Re-treatment was made at as near a 25-day interval as possible (see table 6), so that all treatments might be compared on the same basis. Table 6 presents details of treatments and the percentage of the crop infested at harvest. Infestation at harvest shows good commercial control of walnut husk fly in 1957 by practically all treatments. In summary, average percentage infestation at harvest for the 2-year period for each treatment type with malathion was as follows: no treatment, 7 trials (37%); aircraft bait spray, 5 trials (1%); air-blast sprayer bait spray, 7 trials (2%); full-coverage sprays, 4 trials (2%); bait-type spray coverage (air-blast sprayer) without bait, 3 orchards (5%). It will be noted that single applications of bait sprays were applied in two of the trial orchards in 1957 (orchards 7 and 13, table 6) with a good control in each case. The first of these (orchard 7) had a light carry-over from the previous season, but orchard 13 was untreated in 1956. In this orchard, significant flights did not

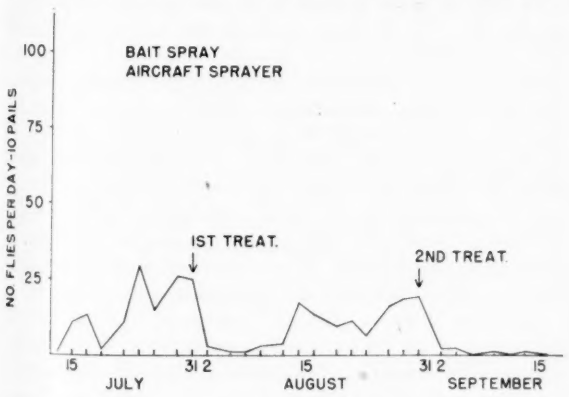

FIG. 3.—Average bait-pail catches per day of walnut husk flies in two Eureka walnut orchards (9 and 10, table 6) in relation to time of treatment with bait sprays applied by aircraft sprayer in 1957.

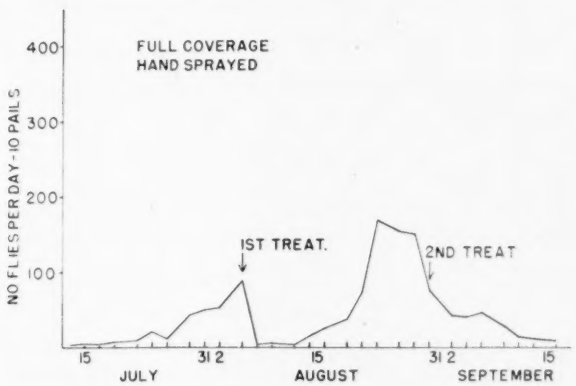

FIG. 5.—Average bait-pail catches per day of walnut husk flies in three Eureka walnut orchards (4, 5, and 6; table 6) in relation to time of treatment with full-coverage (hand-sprayed) malathion sprays, in 1957.

Table 5.—Effects of treatments for walnut husk fly on bait pail returns in Eureka walnut orchards, 1957.

Orchard No. and Treatment[a]	Within Orchards: Per Cent Reduction, 1st 10 Days[b] After Treatment as Compared with 10 Days Before Treatment		
	1st treatment	*2nd treatment*	*Average*
C. 8, 9 Aircraft bait spray (Staley No. 7, malathion)	92	92	92
D. 10, 11, 12 Ground equipment bait spray (Staley No. 7, malathion)	94	80	87
B. 4, 5, 6 Full coverage (Malathion)	88	61	74
E. 14, 15 Bait-type spray, no bait	2	68	35
	Per Cent Increase		
A. 1, 2, 3 Check, untreated	80	15	

[a] Letter and number code refer to orchards listed in table 6. See table 6 for details of treatments.

[b] Data refer to flight within orchards after application of listed treatment as compared to before treatment. Since flight in check orchards increased steadily, the per cent reduction in treated orchards is actually higher than indicated.

occur until the third week in August (see fig. 7). A single bait spray timed to coincide with onset of oviposition effected good control (fig. 7 and table 6).

The consistent performance of bait sprays in controlling walnut husk fly offers promise in this respect. However, in 1957, a spectacular increase in populations of *Tetranychus cinnabarinus* (Boisd.)[4] occurred in orchards of the Placentia variety, and on Placentia and Payne trees interplanted in Eureka orchards, which received bait sprays (malathion-corn protein hydrolysate) whether by aircraft or air-blast sprayers. This upsurge in mite populations was presumably related to the effects of a low dosage of malathion applied in bait-type spray coverage upon

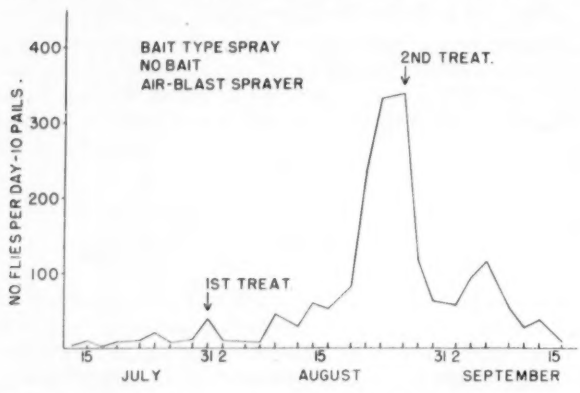

FIG. 6.—Average bait-pail catches of walnut husk flies per day in two Eureka walnut orchards (14 and 15; table 6) in relation to time of treatment with bait-type spray applications by air-blast sprayer of malathion (protein hydrolysate bait omitted) in 1957.

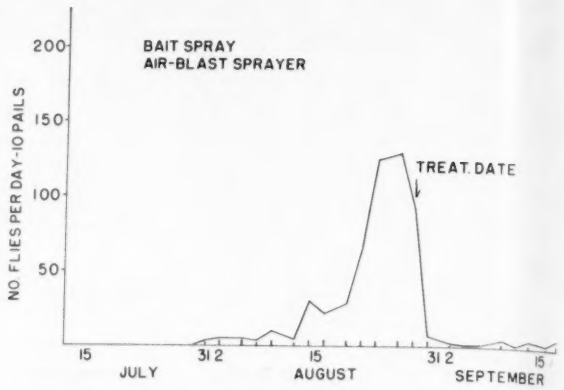

FIG. 7.—Average bait-pail catches per day of a late-emerging population of walnut husk flies in a Eureka walnut orchard (13, table 6) treated with one application of bait spray by air-blast sprayer resulting in commercial control.

predators of this mite. Examples of the effect of bait sprays upon mite populations follow.

In a Placentia orchard contiguous with a Eureka orchard in which a bait-spray experiment was being carried out, 5 out of 12 rows of Placentia trees were treated with a bait spray on August 1 (see orchard 11, table 6, for treatment details). By August 26, these five rows had been severely defoliated by *T. cinnabarinus*, while the remaining rows (untreated) had only light foliage injury from this mite. The Eureka orchard treated in the same manner had only a trace mite population. Apparently, the Eureka variety is relatively resistant to this mite, as compared with the Placentia and Payne varieties, as observations recounted below will further attest.

At another location, 5 adjacent acres of Placentias were treated with a bait spray on August 1, along with the 5 acres of Eurekas in the same planting (orchard 10, table 6). The Placentias became severely infested with *Tetranychus cinnabarinus*. A few Eureka trees were moderately infested. Eight oz. of demeton per acre was added to the second bait spray applied by air-blast sprayer. This treatment very effectively controlled the mite infestation for the remainder of the season.

In two orchards treated with bait sprays by aircraft (orchards 7 and 9, table 6), Placentia or Payne walnut trees interplanted in the Eurekas became severely infested by mites, and these spread to some extent to Eurekas causing light to moderate infestation. In a third orchard (orchard 8, table 6) no mite infestation developed on interplanted Placentia trees. In addition, two solid Placentia plantings treated by aircraft bait sprays developed severe leaf injury from *T. cinnabarinus*, while an untreated orchard in the same area remained free of significant mite populations. Where solid blocks of Eurekas were treated, there was no significant mite problem.

At least in some seasons, bait-spray applications of malathion result in high mite populations. This readily suggests the selection, if possible, of another toxicant for bait-spray use against walnut husk fly which would not have this effect, or the selection of an acaricide to include with bait sprays. Studies are underway along these lines.

[4] Identified by A. E. Pritchard.

Table 6.—Per cent Eureka walnuts infested by walnut husk fly at harvest following described treatment, 1957.

TREATMENT AND ORCHARD NO.	LOCATION	ACRES	EQUIPMENT	MATERIALS/ ACRE	GALLONS WATER/ ACRE	TREATMENT DATES	PER CENT NUTS INFESTED
A. Check							
1	Sunnymead	15	Untreated				47.9
2	Calimesa	5	Untreated				51.2
3	Yucaipa	2	Untreated				11.1
B. Full Coverage-Hand Sprayed							
4	Calimesa	3	Standard sprayer[a]	Malathion 25% W.P. 8 lbs.	700	8/5, 8/30	4.3
5	Puente	5	Standard sprayer	Malathion 25% W.P. 9 lbs.	600	7/30, 8/26	0.0
6	Yucaipa	2.2	Standard sprayer	Malathion 25% W.P. 8 lbs.	700	8/5, 8/30	0.7
C. Bait Spray—Aircraft							
7	Puente	45	Aircraft	Malathion 25% W.P. 4 lbs. Staley No. 7, 2 qts.	10	7/31	2.3
8	Beaumont	14	Aircraft	Malathion 25% W.P. 4 lbs. Staley No. 7, 2 qts.	10	7/31, 9/1	0.2
9	Sunnymead	10	Aircraft	Malathion 25% W.P. 4 lbs. Staley No. 7, 2 qts.	10	7/30, 9/1	0.0
D. Bait Spray							
10	Yucaipa	5	Spray duster[b]	Malathion 25% W.P. 4 lbs. Staley No. 7, 2 qts. (Second spray same+Systox, 1 qt.)	100	8/1, 8/27	3.6
11	Spadra	9.4	Speed sprayer[c]	Malathion 25% W.P. 4 lbs. Staley No. 7, 2 qts.	100	8/1, 8/28	1.9
12	Calimesa	3.6	Spray duster	Malathion 25% W.P. 4 lbs. Staley No. 7, 2 qts.	100	8/1, 8/26	2.2
13	Calimesa	4.8	Spray duster	Malathion 25% W.P. 4 lbs. Staley No. 7, 2 qts.	100	8/28	1.4
E. Bait-Type Spray (No Bait)							
14	Calimesa	4.9	Spray duster	Malathion 25% W.P. 4 lbs.	100	7/31, 8/26	2.0
15	Yucaipa	1	Spray duster	Malathion 25% W.P. 4 lbs.	100	8/1, 9.3	5.8

[a] High-pressure sprayer equipped with tower and two ground leads.
[b] A citrus "spray duster," an air-blast concentrate sprayer equipped with high-pressure pump and low-velocity, high volume No. 5 Master Fan. One-side delivery used.
[c] Model 30 two-side delivery, standard nozzle.

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The Behavior of Agricultural Insects Toward Olfactory Repellents in the Olfactometer and in Split-Arena Tests[1]

W. F. Chamberlain[2]

Abstract

Previous investigations have shown that camphor, camphene, paradichlorobenzene (PDB), acetophenone and sassafras oil are highly repellent to agricultural insects in olfactometer tests, but when these compounds were field-tested they showed only slight, if any, repellency. Split-arena tests also verified the fact that these materials possessed very little repellency to agricultural insects. It was concluded that the failure of the split-arena tests to point out the repellency was due to an unsatisfactory aerial boundary between treated and untreated areas. A similar condition probably existed in the field where foliage was treated with an olfactory repellent. Differences in temperature had little effect on the results of the tests. It is expected that gustatory materials would show more repellency toward agricultural pests than olfactory materials.

Previous investigations have shown that several compounds are highly repellent to agricultural insects as determined by the olfactometer, but when these compounds were field tested they showed only slight, if any, repellency (Thomas & Chamberlain 1959). To resolve this difficulty, further tests were conducted with the olfactometer and attempts were made to establish a satisfactory split-arena test. Correlation was then established between the field observations and the results with olfactometer and split-arena tests. In addition, the effect of temperature on the action of the repellents was investigated.

Procedure.—The olfactometer tests were conducted with the instrument previously described (Chamberlain 1956) except for the following slight modifications of procedure and technique. In these tests a U-tube was used to hold the test chemical and impart it to the air stream. In all the tests with liquid materials, the bottom curved portion of the U-tube was filled with 5-millimeter glass beads. In each test the glass beads were completely saturated with two milliliters of test solution. Free movement of air was maintained with two milliliters of test solution, but if larger quantities were tried the flow of air was blocked. When solid materials were tested, the beads were not used and the curved portion of the U-tube was filled with the test chemical. Only crystalline solids were used as powders tended to pack to such an extent that the flow of air was prevented.

No tests were set up for the specific purpose of determining the effect of temperature on the reaction of the insect, but a record of the temperature variation in the test room was kept and from these records the effect of the temperature change could be evaluated. The temperature was determined during a test using a thermocouple inserted in the test chamber.

Attempts were made to establish a satisfactory split-arena test procedure for the chemicals which had been successfully tested in the olfactometer. The procedures involved the use of 6-inch round embroidery hoops. They were covered on the top or the bottom or both edges of the rim with cheesecloth, depending on the design of the experiment. The $\frac{1}{2}$-inch distance between the top and bottom of the rim supplied a cage which could confine the insects relatively close to the treated surface. Even closer confinement could be obtained by slipping the inside hoop slightly up so that a $\frac{1}{16}$- to $\frac{1}{8}$-inch cage was formed between the bottom cheesecloth and the treated surface. This cage was particularly useful with the active flying insects used in these tests and confined the insects close to the treated surface.

Determinations of the effect of the chemicals on the test insects in the split arena were made by counting the number distributed on or over treated and untreated surfaces. Counts were made at 10, 20, and 30 minutes after initial exposure. In all cases the test repellents were applied to filter paper by dipping in acetone solutions. Before exposing the insects, the treated filter paper was air-dried for several minutes to remove the acetone. In setting up the split-arena tests, care was exercised that replicates were placed so as to average out the possible effect of light direction, air currents, temperature differences, and other secondary influences on the activity of the insects.

The test insects for both the olfactometer tests and the split-arena tests were collected in the field as they became seasonally abundant. After being brought into the laboratory, they were anaesthetized with carbon dioxide and 20 were counted out for each test.

The insects used in the olfactometer tests were the tarnished plant bug (*Lygus lineolaris* (P. de B.)), the honey bee (*Apis mellifera* L.), the three-cornered alfalfa hopper, *Spissistilus festinus* (Say)), the lesser clover leaf weevil (*Hypera nigrirostris* (F.)), and *Hypera meles* Fab., a cloverhead feeding weevil. The split-arena tests were conducted with the tarnished plant bug, *Hypera meles*, and the pea aphid, *Macrosiphum pisi* (Harris).

In the olfactometer tests observations were made every 2 minutes on the number of insects occupying each side of the olfactometer test chamber. These observations were made for a period of 10 minutes. After reversing the flow of air, there was a 5-minute adjustment period and another 10-minute reading period in order to complete a test.

Several chemicals used to repel mosquitoes or other arthropods attacking man and animals were tested (King 1954, Gilbert 1957). These included n-butylacetanilide, dibutyl adipate, normal hexyl mandelate, benzyl benzoate, cyclohexycyclohexanol, and acetophenone. Besides the above repellents, tests were conducted with paradichloro-benzene, camphor, camphene, and sassafras oil.

Results and Conclusions.—The results with the olfactometer shown in table 1 represent the average of four tests. Again, as in previous tests the most volatile ma-

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Table 1.—Results obtained with olfactometer repellency tests.

Material	Dilution Ratio	Air Temperature	Average Amount of Chemical in Mg./Liter/Min.	Average Repellency Index
	Tarnished plant bug			
Sassafras oil	1/3	83.2	0.25	53.6
Camphor	1/7	81.9	.32	25.8
	1/3	83.5	.164	20.6
Camphene	1/7	81.9	1.12	8.7
	1/3	82.0	1.10	19.4
Butylacetanilide	1/7	80.2	.024	8.5
	1/3	80.5	.090	24.5
Dibutyl adipate	1/7	81.5	.080	4.2
	1/3	82.0	.03	0.5
Hexyl mandelate	1/7	80.5	.046	4.7
	1/3	84.0	.066	15.1
	1/3	84.1	.118	13.2
	Lesser clover leaf weevil			
PDB	1/7	68.5	0.75	10.0
	1/7	76.9	.98	59.2
	1/3	76.9	1.40	66.8
	1/3	81.0	1.44	59.1
Camphene	1/3	76.1	2.84	63.4
Dibutyl adipate	1/3	80.0	.052	3.2
Hexyl mandelate	1/7	84.5	.025	30.0
	1/3	80.0	.061	30.0
	Three-cornered alfalfa hopper			
Sassafras oil	1/7	82.0	0.145	29.9
Camphor	1/7	82.0	.90	60.8
	1/3	81.0	.65	57.6
Acetophenone	1/7	77.9	.47	60.8
	1/7	79.9	.37	66.2
	1/3	83.2	.76	62.8
Dibutyl adipate	1/7	77.0	.091	0.8
	1/3	79.4	.258	3.8
	Hypera meles			
PDB	1/3	70.7	1.36	59.0
	1/2	80.0	2.06	84.5
Camphene	1/7	72.0	3.30	21.8
	1/3	71.1	4.60	47.5
	1/2	71.6	4.00	49.8
	Worker honey bees			
Sassafras oil	1/3	77.2	0.117	44.1
Acetophenone	1/7	78.0	.315	42.2
Benzyl benzoate	1/7	78.1	.37	10.4
	1/3	73.0	1.14	5.0
Cyclohexylcyclohexanol	1/7	73.5	.39	15.1
	1/3	74.5	.67	32.2

terials with the highest concentration in the air stream were in general the most repellent. Among the chemicals tested the most repellent were camphor, camphene, PDB, acetophenone, and sassafras oil. This is particularly true if no reference is made to the concentration. On a concentration basis, however, some of the other materials might be considered more repellent. As an example, cyclohexylcyclohexanol at 0.39 mg. per liter of air per minute had a repellency index of 15.1; at 0.67 mg. per liter of air per minute the repellency index was 32.2. On this basis, if the concentration were increased to the 0.90 mg. per liter of air per minute (the maximum concentration of camphor) the repellency could be expected to go up to an index of 45. Even higher concentrations of camphene are recorded in the table, and repellency index of 50 or greater for cyclohexylcyclohexanol at 1.0 mg. per liter of air per minute could be easily justified.

The fact that sassafras oil was rated in this group was surprising. On selecting this material it was expected that there would be little repellency but that in all probability there might be an attraction. It may be that this again illustrates the general rule that all odorous materials which are attractive eventually become repellent if the concentration is increased enough (Dethier 1947). No attempt was made in these tests to lower the concentration of sassafras oil below that given in the table.

The results with camphor are interesting from another standpoint. It was noted in tests with the three-cornered alfalfa hopper and with the tarnished plant bug that the repellency is the inverse of what would be expected from the dilution ratio. At 1 part odorized air to 7 parts unodorized air, the repellency was greater, but only slightly greater than when there was 1 part odorized air to 3 parts unodorized air. No explanation can be offered for this effect except that camphor was one of two crystalline materials tested. PDB, the other crystalline material, also showed unexplainable differences in the index values. Possibly this indicates a difficulty in the use of crystalline materials in the olfactometer. Differences in temperature cannot be responsible as in all the tests with camphor there was no appreciable difference in the temperature.

There was also a closer correlation between the amount of chemical in the air stream than to the dilution ratio. In several instances where the dilution was greater, a higher repellency was shown, but if the amount of chemical is noted these discrepancies resolve themselves in most cases.

Of the other materials tested, cyclohexylcyclohexanol might be considered as slightly better than hexyl mandelate. These two materials, along with the other repellents for blood-sucking arthropods, belong in a second natural group of less value than the first group.

The results obtained in the series of tests with camphene compare very favorably with the previous tests (Chamberlain 1956). Without considering the differences due to different species of insects, the results with the U-tube are approximately those obtained with the jar-type odorizer. The concentration of the camphene in the air stream is of the same order, being only slightly higher with the jar method. Since the jar method requires about 50 ml. of the test chemical, the U-tube has a definite advantage where only small quantities of a test repellent are available.

In general the different insect species did not behave differently toward a repellent. Especially is this true if differences in concentration between tests are considered. The one material that did exhibit marked differences in effect on two species was hexyl mandelate. The average repellency index for the tarnished plant bug was ap-

proximately 11, with no consistent effect of concentration, and for the lesser clover leaf weevil the value was 30, again without effect of concentration. In this instance the concentration of the hexyl mandelate was actually slightly higher in the tarnished plant bug tests. When a single material, such as camphene, was compared on different insects, the same general lack of difference was noted except when the concentration was changed. In the tests with *Hypera meles* and the lesser clover leaf weevil, the concentration was in the order of 3 or 4 mg. per liter of air per minute and the index was generally in the order of 50 to 60. When the concentration dropped to 1 in the tests with the tarnished plant bug, the index of repellency fell below 20. In one test with *Hypera meles*, at 3.3 mg. per liter of air per minute an index value of 21.8 is recorded. This value is unexplainably lower than expected but it is in the right direction when compared with the other tests with *Hypera meles* and it should not invalidate the general conclusion.

In the tests reported in this paper, no particular attempt was made to control the temperature accurately except to prevent the temperature from rising about 85° F. This lack of temperature control was in part due to lack of equipment for accurate temperature control. A more significant reason was that it was important to determine if the olfactometer equipment could give good results when the temperature varied within reason. If the tests are taken as a whole, it may be seen that variations of 5 to 8 degrees did not have any effect on the outcome of a test. The only instance where a marked difference in repellency might be attributed to a difference in temperature was the tests with PDB on the lesser clover leaf weevil. There a difference of approximately 9 degrees might be related to the index of 10 at the lower temperature and an index of 59 at the higher temperature. Even this great a difference needs further testing in order to be sure some other factor was not involved. PDB did not show this temperature effect on *Hypera meles*.

The results of the split-arena tests are shown in table 2. The tests were conducted in several ways to determine a procedure which would most accurately represent the true measure of repellency. Test 1, series 1, was conducted with *Lygus lineolaris*. Four test arenas were used, set at 90° to each other, and a total of 12 readings was made. The insects were in direct contact with the filter paper and in a cage one-half inch deep covered with cheesecloth. The results recorded show that only slightly more than 50% remained on the untreated portion of the test arena.

In test 1, series 2, an attempt was made to confine the insects as closely as possible to the treated surface. The cage in this test was no more than one-eighth inch deep with a cheesecloth cover. Otherwise the test conditions were the same as in test 1, series 1. The close confinement evidently had no effect on the result, as still only slightly more than 50% remained on the untreated surface. When the concentration of the camphor was increased to 10% in test 2, slightly less than 50% preferred the untreated portion, but the difference was not significant.

Test 3 was conducted with *Hypera meles* to determine if another insect would respond more satisfactorily. The test conditions were the same as in test 1, series 1, except 2 cages were placed at 180° to each other. The test was repeated a day later. The result was again an approximately equal distribution of the insects. Apparently different species would not result in the expected distribution of the insects.

Test 4 was conducted with camphene instead of camphor to determine if the failure to obtain repellent action was due to the test chemical. The test conditions were again the same as in the first test, but the change to a new chemical had no effect on the result.

Test 5, series 1, was conducted with *Macrosiphum pisi*. A single test was set up on 5 consecutive days. On the first day 80% of the insects remained on the untreated side of the test arena, but upon completion of the five tests the average had dropped to 58.7%.

In test 5, series 2, the insects were confined in a cage one-eighth inch above the treated surface. In this test the insects were not in direct contact with the treated filter paper. An additional difference between test 5, series 2, and previous tests was the use of filter paper divided into quarters so that there were two treated sections placed directly opposing. The other conditions were the same as in the first test. The distribution still remained about equal between treated and untreated portions. In the final test acetophenone was employed as in test 1, series 1, but in general no change resulted.

The general conclusion reached from the split-arena tests was that very slight if any repellency could be demonstrated by materials that had shown olfactory repellency in the olfactometer. The split-arena tests served a second purpose which at first was not obvious. The three materials that were tested in these tests are in all probability not gustatory repellents. If these materials were gustatory repellents, the insects would have surely shown more marked repellency when in physical contact with the treated surface. The air dilution effect of olfactory repellents would be at a minimum in the case of a gustatory repellent. If this is the case, then camphor, camphene, and acetophenone are only olfactory repellent materials as indicated by the olfactometer tests where a gustatory effect would be at a minimum.

The split-arena tests help to explain the conflicting results obtained with the olfactometer and in field experiments. It had been previously observed that, in tests with *Macrosiphum pisi* chemicals such as camphene, methyl pentanone, and paradichlorbenzene, which exhibited a high repellency in the olfactometer, did not materially repel the aphids in the field (Thomas & Chamberlain

Table 2.—Results of split-arena repellency tests.

Test No.	Material and Concentration	Test Insect	Per Cent Insects on Untreated Side	
			Series 1	Series 2
1	Camphor—1%	*Lygus lineolaris*	55.4	54.2
2	Camphor—10%	*Lygus lineolaris*	47.0	
3	Camphor—1%	*Hypera meles*	51.1	
4	Camphene—1%	*Lygus lineolaris*	52.4	
5	Camphene—1%	*Macrosiphum pisi*	58.7	51.7
6	Acetophenone—1%	*Lygus lineolaris*	49.2	

1959). A possible explanation was that the counts in the field tests were made after a day's lapse between application and sampling.

The split-arena tests offer another explanation for the conflicting results. Since the counts on the split arena were made at very short intervals of time, there can be no doubt that the chemicals were still present in the treated filter paper; yet no material repellency could be demonstrated by the split-arena technique. The odor of the chemicals was obvious at the end of each test. These results could be explained if we assume that in the split arena there exists no sharp boundary in the air above the treated and untreated portions of the arena. In the olfactometer such a sharp boundary does exist. The insects encountering the rather ill-defined boundary in the arena cannot distinguish the treated from the untreated areas. With very low concentrations the air boundary would shift over the treated surface while at high concentrations the boundary would shift over the untreated surface, and in fact the whole cage might in a sense become treated. This may explain the field results. The actual aerial concentration of the repellent is a doubtful value. The insect may certainly be able to detect these concentrations, but because of ill-defined boundaries which exist even within a treated area they are unable to distinguish the treated from untreated foliage. Actually shifting boundaries may exist in the field due to air currents.

The results of the split-arena tests when compared with the olfactometer tests and field observations may mean that an olfactory repellent will have a limited area of agricultural application. Possibly more effort should be directed toward the finding of a satisfactory gustatory repellent. Since gustatory repellents could be very nonvolatile, they should remain on the plant in sufficient concentration for a much longer period of time. Since generally higher concentrations are needed for gustatory repellency, a great deal of investigation will be needed to find this type of repellent acting in a sufficiently low concentration.

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Persistence of Some Chlorinated Hydrocarbon Insecticides in Turf Soils[1]

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Abstract

To determine the residual effectiveness of some chlorinated hydrocarbon insecticides for the control of Japanese beetle grubs (*Popillia japonica* Newm.), turf plots were treated at various concentrations in 1946 with chlordane and BHC, in 1949 with heptachlor and in 1954 with aldrin. The insecticides were applied as top dressings to the established turf and the experimental plots were left undistrubed throughout the years. In 1957 and 1958 soil samples were collected from the upper 3″ soil layer.

An average of 15% of the applied chlordane was recovered 12 years after treatment as estimated by a specific chemical analysis and an average of 12% as estimated by bioassay.

Eleven years after treatment, BHC was recovered to an extent of 41% of the applied dosage as estimated by chemical analysis and to an extent of 8% as measured by bioassay.

No heptachlor was recovered from soils which had been treated with heptachlor 9 years earlier, when analyzed by a specific colorimetric method. However, using a bioassay procedure, 4 to 5% of the applied toxicant was found. This toxicant proved to be heptachlorepoxide.

Most of the aldrin had disappeared during a period of 4 years. However, part of the aldrin had been converted into dieldrin, which was recovered to an extent of 8 to 10% of the applied dosage.

Aldrin, which had been rototilled into the soil immediately after application, persisted considerably longer, though the rate of dieldrin formation was more or less the same as compared with soils which had not been rototilled after treatment.

During recent years insecticides have been found more and more in various soils, due to direct soil treatment or to applications of sprays to agricultural crops. Residue data of insecticides in soils by various workers (Chisholm *et al.* 1951, Fleming *et al.* 1951, Ginsburg & Reed 1954, Ginsburg 1955, Lichtenstein 1957, Lichtenstein & Schulz 1959), indicated that in some cases the disappearance of insecticidal residues was unable to keep up with application and resulted in an accumulation of a particular insecticide in soils.

So far, residue data have been obtained primarily from soils which had received repeated application over a period of years, or only one single application at the beginning of the experiment. In the latter case, residue data available cover a rather short period of 4 to 6 years. But

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in most of those experiments soils were either used for crop responses to the insecticides and cultivated or, at least, disked or rototilled in order to secure weed control.

It would, therefore, be of interest to know how long a certain insecticide persists in a soil, which had received one single application only and which had not been cultivated over a relatively long period of time. Turf plots, which fulfilled those requirements, were available from two cemeteries and two park areas in Ohio. By using a bioassay procedure for estimating residual toxicity and a specific chemical analytical method for measuring a particular insecticide, information should be obtainable concerning the persistence of the insecticide as well as the formation of breakdown products of the original toxicant. Soil samples were collected and analyzed by chemical and bioassay methods.

PROCEDURE.—*Treatments.*—In order to determine the residual effectiveness of some chlorinated hydrocarbon insecticides for the control of Japanese beetle grubs turf plots were treated with various insecticides.

Chlordane emulsion was applied on September 6, 1946 to 10′×10′ turf plots (park area), consisting of a sandy loam (Cleveland, Ohio). Application rates were 1.0, 5.0, 15 and 25 lbs./acre, each treatment was replicated five times.

Benzene hexachloride (BHC) was mixed with Milorganite (activated sludge marketed in dry granular form by the Milwaukee sewage disposal plant and used as a fertilizer) and applied on September 26, 1946 with a 12-inch fertilizer spreader to turf plots (sandy loam, Cleveland, Ohio), which had a size of 10′×10′. Rates of application were 0.25, 2.50, 5.00 and 10.00 lbs./acre of actual gamma isomer, each treatment was replicated five times.

Heptachlor was applied on June 8, 1949 to turf plots (silty clay loam) in a cemetery (Wellsville, Ohio). The insecticide was applied in the same way as the BHC. Application rates were 1.0, 5.0, 10.0 and 20.0 lbs./acre, each treatment was replicated five times.

Aldrin was applied on May 13, 1954 to turf plots (silty clay loam) in a cemetery (New Matamoras, Ohio) according to the same method as described for BHC. Application rates were 3.0 and 75.0 lbs./acre, each treatment was replicated three times.

None of the insecticides applied were worked into the soil and the experimental plots were left undisturbed throughout the years.

Sampling.—Soil samples from the chlordane-treated plots were collected on May 5, 1958 (12 years after treatment), from the BHC-treated plots on April 1, 1957 (11 years after treatment), from the heptachlor-treated plots on April 28, 1958 (9 years after treatment) and from the aldrin treated plots on May 14, 1958 (4 years after treatment).

Twelve cores ($\frac{3}{4}$ inch diameter–3 inches long) were collected with a soil auger from each of the five replicates of chlordane- and heptachlor-treated soils amounting to a total of 60 cores for each treatment.

Ten cores were collected from each of the five replicates of the BHC-treated plots, amounting to a total of 50 cores for each treatment, and 20 cores were collected from each of the three replicates of the aldrin-treated plots, amounting to a total of 60 cores for each treatment.

The 50 or 60 cores collected from the plots having received the same treatment, were mixed and submitted for analysis.

ANALYTICAL METHODS.—*Chemical analyses.*—Before each extraction, the total soil sample was screened and mixed on a sheet of paper (30″×30″) by rolling in different directions. After that, 400 grams of field moist soil were placed into 2-qt. wide mouth Mason jars together with 200 grams anhydrous sodium-sulfate. An additional 100 grams of soil were dried for 24 hours at 46° C. to determine the dry weight of the soil.

The extraction solvents used were a mixture of colorimetric pentane and acetone (4 to 1), for heptachlor, heptachlorepoxide and chlordane, and a mixture of redistilled hexane and isopropanol (2 to 1) for aldrin and dieldrin. Two milliliters of solvent were used per gram of wet soil and a 1-hour head to end tumbling was applied. After tumbling, the supernatant liquid was decanted through filter paper and the recovery volume was recorded, to be used as a factor for calculating the results.

When heptachlor, heptachlorepoxide and chlordane were extracted, the jars containing soil and solvent were placed—after tumbling—into a refrigerator for one-half hour, in order to minimize the evaporation of pentane while filtering.

The isopropanol or the acetone were removed from the soil extracts by washing first with water and then with a saturated solution of sodium chloride. The acetone or alcohol free phase was then dried over anhydrous sodium sulfate.

Extracts from aldrin-treated soils were analyzed for both aldrin and dieldrin. The clean-up of the extracts as well as the separation of aldrin and dieldrin was done according to the procedure as described by Lichtenstein & Schulz (1959). Analyses were made according to O'Donnel *et al.* (1954, 1955).

Extracts from heptachlor-treated soils were analyzed for heptachlor and heptachlorepoxide. The extract was concentrated to about 25 ml., to which 0.5 grams of "Nuchar" activated carbon (C 190-N, pH 6) was added. The mixture was swirled gently for 1 minute. After an additional 5 minutes, it was filtered through a half inch layer of asbestos with some glass wool on top. Chromatographic columns (7″×$\frac{3}{4}$″), with sintered glass disk bottoms, were used. After several washings, the clear eluate was concentrated in a 50° C. water bath and then added to a 10-gram florisil (60×100 mesh) column (20 mm. diameter). Heptachlor was eluted by adding 80 ml. of colorimetric pentane. The remaining heptachlorepoxide could be washed out from the column with 100 ml. of colorimetric pentane-acetone (6%). Heptachlor and heptachlorepoxide fractions were analyzed according to the Polen-Silverman method (Polen & Silverman 1952, Ordas *et al.* 1956).

The violet color developed for heptachlor was measured photometrically at 567 mμ, and the yellow color developed for heptachlorepoxide was measured at 410 mμ.

Extracts containing chlordane were concentrated and then cleaned up by passing through a 10-gram florisil (60×100 mesh) column. Analyses were made according to Ordas *et al.* (1956).

Soils which had been treated with BHC were analyzed according to the method of Schechter & Hornstein (1952). A change of the method (Lichtenstein *et al.* 1956) elimi-

nated a special extraction procedure and permitted the determination of BHC directly in soils.

Each analysis was run in duplicate using a soil blank for the determination of apparent insecticide. In addition, known amounts of insecticide were added to insecticide-free soil samples. This permitted a check for the analytical procedure for each analysis made, Results were expressed in parts per million based on the dry weight of the soil under investigation and in pounds per 3-inch acre, after the dry weight of a 3″ acre had been calculated.

Bioassay.—Drosophila melanogaster Meig. was used as a test insect and a direct feeding method (Edwards *et al.* 1957) was employed. This eliminated a special extraction procedure, which to some extent influences the accuracy of the analysis.

Soils treated with heptachlor, though containing at the time of the analyses heptachlor and heptachlorepoxide, were measured against a heptachlor standard. Soils treated with aldrin were measured against an aldrin as well as a dieldrin standard.

RESULTS AND DISCUSSION.—The analytical results as obtained by chemical analyses and bioassays are summarized in table 1. Comparing the per cent recoveries of the applied insecticide dosages as estimated by both methods, a certain discrepancy is obvious. Two groups of insecticides were differentiated: when chlordane and BHC were analyzed, the results obtained by bioassays were lower than the ones obtained by chemical analyses. Quantities estimated by bioassay of soils having been treated with chlordane at the rate of 5, 15 and 25 lbs./acre amounted to an average of 65% of that obtained by chemical analysis. When soils treated with BHC were analyzed, estimates obtained by bioassay amounted to only 19.5% (average) of the ones obtained by chemical analyses.

Since a bioassay procedure measures residual toxicity and the colorimetric analysis a specific compound only, this might indicate that the originally applied insecticide broke down to a nontoxic compound, which was still detected by the colorimetric analysis. For all practical purposes, the bioassay results seem to give a more realistic assessment of the insecticidal or contaminative residues.

Heptachlor and aldrin were found to belong to the second group of insecticides, where estimates of residues obtained by bioassays were considerably higher than the ones obtained by chemical analyses. In this case, apparently, a compound was formed which was not detected by the specific colorimetric methods for either heptachlor or aldrin, yet was still toxic to insects. No heptachlor was recovered by chemical analysis from soils which had

Table 1.—Recoveries of insecticide residues from turf plots treated with chlordane and BHC in 1946, heptachlor in 1949 and aldrin in 1954.[a]

Insecticide	Amount Applied (Lb./A.)	Recovered					
		Chemical Assay			Bioassay		
		P.p.m.	Lb./3″—Acre	Per Cent of Applied	P.p.m.	Lb./3″—Acre	Per Cent of Total Applied
Chlordane	1.0	0.17	0.17	17.0	0.21	0.21	21.0
	5.0	.65	.64	12.9	.45	.44	8.9
	15.0	1.89	1.86	12.4	1.27	1.25	8.3
	25.0	4.50	4.42	17.8	2.63	2.59	10.3
BHC[b]	0.25	0.09	0.09	35.4	0.00	0	0.0
	2.5	.92	.91	36.2	.20	0.19	7.9
	5.0	2.02	1.98	39.6	.50	4.90	9.8
	10.0	5.25	5.13	51.3	1.42	1.40	14.0
			Heptachlor				
Heptachlor	1.0	0	0	0	0.04[c]	0.04	3.6
	5.0	0	0	0	.19[c]	.17	3.4
	10.0	0	0	0	.42[c]	.38	3.8
	20.0	0	0	0	1.32[c]	1.17	5.9
			Heptachlorepoxide				
Heptachlor	1.0	0.07	0.06	6.4			
	5.0	0.24	0.21	4.3			
	10.0	0.41	0.37	3.7			
	20.0	1.82	1.62	8.1			
			Aldrin				
Aldrin	3.0	0	0	0	0.16[d]	0.14	4.6
	75.0	0.13	0.11	0.15	4.52[d]	3.91	5.2
			Dieldrin				
Aldrin	3.0	0.36	0.31	10.4	0.29[e]	0.25	8.4
	75.0	7.23	6.22	8.3	8.53[e]	7.42	9.9

[a] Samples were taken in 1958 as follows: Chlordane, 12 years after application to sandy loam soil; heptachlor, 9 years after application to silty-clay loam soil and aldrin 4 years after application to silty-loam soil; samples were taken in 1957 from sandy loam soil, 11 years after BHC was applied.
[b] Gamma isomer.
[c] Against heptachlor standard.
[d] Against aldrin standard.
[e] Against dieldrin standard.

been treated 9 years earlier with heptachlor. However, from 3.6 to 5.9% of the insecticide applied was recovered by bioassay, when measured against a heptachlor standard. It was found that the toxicant present was heptachlorepoxide (table 1). Since LD-50 values for *Drosophila* were found to be lower for heptachlor as compared with heptachlorepoxide, the results obtained by bioassay are probably somewhat higher than those given in table 1. During the 9 years in which the insecticide was on or in the soil, heptachlor had been converted to some extent to its epoxide which was found to be the only toxicant present after that time.

When aldrin-treated soils were analyzed, no aldrin was recovered from the plots that had been treated at a rate of 3 lbs./acre as estimated by chemical procedures. Yet 4.6% of the originally applied toxicant was recovered by bioassay. The plots that had been treated at a rate of 75 lbs./acre contained, 4 years later, 0.15% of the applied aldrin as measured by chemical analysis, and 5.2% as measured by bioassay against an aldrin standard. This seemed to indicate that part of the originally applied aldrin had been converted into another toxicant, which was not detected by the phenylazide method for aldrin, but showed up in the bioassay procedure. This toxicant proved to be dieldrin (tables 1 and 2). Since the major part of the residues recovered from the aldrin-treated soils was dieldrin, results obtained by bioassay—estimated against a dieldrin standard—were similar to those obtained by chemical analyses.

It was previously reported (Lichtenstein 1957) that DDT applied to turf plots in 1945 was recovered 10 years later to an extent of 11 to 18% of the applied dosages in the upper 6″ soil layer. In this study an average of 15% of the applied chlordane was recovered after 12 years, as measured by chemical analysis and to an extent of 12% as measured by bioassay. An average of 40.6% of the applied BHC was recovered 11 years after treatment as measured by chemical analysis and only 7.9% as estimated by bioassay. An average of 5.6% of the applied heptachlor was recovered after 9 years in the form of heptachlorepoxide. Four years after treatment with aldrin, only 0.08% of the applied dosage was recovered as aldrin, but 9.4% of the applied dosage was found in the form of dieldrin in the upper 3″ layer of the treated soil.

As the insecticides were applied in Ohio to turf and the soil surface only, a comparison was made with residue studies in Wisconsin (Madison), where the insecticide was rototilled into the soil immediately after application (table 2). In the Wisconsin plots, which were rototilled or disked periodically, aldrin had been applied to a similar soil type (Miami silt loam) and samples were also collected 4 years after treatment.

Table 2.—Recoveries of aldrin and dieldrin residues from silty loam plots 4 years after treatment with aldrin.

Amount Applied (Lbs./Acre)	Type of Application	Locality	Amount Recovered			D[a]/A
			P.p.m.	Lbs./3 Inch Acre	Per Cent of Applied Aldrin	
75	Top dressing	Ohio	A[b] 0.13	0.11	0.15	55.7
			D[c] 7.23	6.22	8.25	
				lbs./6″ acre		
20	Worked into soil (5″–6″)	Wisconsin	*A* 0.14	0.22	1.11	6.0
			D 0.84	1.34	6.72	
200	Worked into soil (5″–6″)	Wisconsin	*A* 3.69	5.91	2.94	1.37
			D 5.02	8.08	4.04	

[a] $\frac{D}{A}$ = Ratio $\frac{\text{Dieldrin}}{\text{Aldrin}}$.

[b] A = Aldrin.

[c] D = Dieldrin.

It was found that the amounts of aldrin recovered in both locations were considerably different. Only 0.15% of the originally applied aldrin (75 lbs./acre) was recovered in the soil, to which the insecticide had been deposited on the surface only. But 1.1 to 2.9% of the applied aldrin (20 and 200 lbs./acre) was recovered in the Wisconsin plots. On the other hand, the differences in dieldrin recoveries were relatively small. In Ohio, 8.25% of the applied aldrin was found to be dieldrin as compared with dieldrin recoveries of 6.7 and 4% of the applied aldrin in Wisconsin.

This seems to indicate that aldrin disappears faster when applied to the soil surface only. Once converted into dieldrin, the insecticide seems to be more persistent. This most probably explains why the amount of dieldrin recovered in Ohio was 55.7 times larger than the amount of aldrin found, whereas in Wisconsin only 6 and 1.4 times more dieldrin than aldrin was recovered.

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Recognition of Three Biotypes of the Pea Aphid from Southern Quebec[1]

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ABSTRACT

When three lines (R1, R2 and R3) of the pea aphid (*Macrosiphum pisi* (Harris)) were reared simultaneously for 9 days at 69° F. each on one susceptible (Perfection) and two resistant (Pride and Onward) varieties of peas, *Pisum sativum* L., the lines differed in mean weights and numbers of females produced. R1, R2 and R3 females averaged respectively 3.4, 2.6 and 2.0 mg. on Perfection and 2.4, 1.4 and 1.0 on Onward; the mean numbers of progeny per three females were 192, 134 and 101 on Perfection and 75, 20 and 35 on Onward. Overactivity in adults maintained on Pride and Onward was apparently associated with undernourishment. However, nymphs born on the resistant varieties reached the adult stage without any sign of overactivity.

In 1956, a research program was set up at St. Jean to secure precise information on the biological relationships involved in varieties of peas, *Pisum sativum* L., resistant and susceptible to the pea aphid (*Macrosiphum pisi* (Harris), Homoptera: Aphididae). The aphid stock collected from different localities was apparently not homogeneous for the character of size of the adults. The possibility of biotypes in the stock cultures required that their identity be investigated in order to measure and develop resistance of peas to the more virulent biotypes of the species. Cartier (1957) obtained first evidence of races of the pea aphid in southern Quebec by rearing two lines of apterous viviparous females for 44 consecutive generations. The lines differed in mean weight of the adults and this character was maintained from generation to generation. This is a report on the recognition of three biotypes of the pea aphid from southern Quebec and some of the properties of antibiosis of the pea varieties Pride and Onward.

MATERIALS AND METHODS.—The three lines originated from three apterous viviparous females collected on alfalfa in September. Two (R1 and R2) were started with the largest (3.5 mg.) and the smallest (0.7 mg.) individuals of a population of 60 adults taken at St. Jean, in 1955. Another one (R3) was a line from Waterloo, Que., producing the smallest adults in a group of 35 lines obtained from several localities, in 1956. Before testing, the lines were maintained till March 1957 on Perfection seedlings grown in 6-inch flower pots.

The cage consisted of a sleeve of white nylon marquisette supported by a cylindrical wire frame 20 inches high. The frame was soldered to a sheet metal base ring 5 inches in diameter and 2 inches high. The ring was buried 1 inch into the soil to hold the cage in place. A rubber band secured the sleeve to the upper half of the ring.

The experiments were carried out in a greenhouse compartment provided with thermostatic control of the heating and ventilating systems. A mean temperature of 69° F. and a photoperiod of 16 to 18 hours were adopted as standard conditions. The testing procedure involved the simultaneous rearing under cages of the three lines on three varieties replicated four times. Each cage contained at the beginning three 48-hours-old apterous females on three pea plants. The rate of reproduction was measured by plotting the cumulative numbers of progeny at regular intervals. On the ninth day, some of the progeny became parturient and 10 of each line were taken at random in the four replicates of each variety and weighed individually with a Roller-Smith micro-torsion balance. The significance of the differences in numbers of progeny and average weight was determined by Student's *t* test.

RESULTS AND DISCUSSION.—The differential reactions of the three lines are illustrated in figure 1. On each variety the rates of reproduction of the three lines differed significantly except for R2 and R3 on Pride; the number of progeny of R2 on Onward was less than that of R3, a reversal of the situation in the other two varieties. As shown in figure 2, the weight of adults in the progeny of each line also differed according to the variety of pea. The weight-progeny relationships showed clearly that the three lines of aphids were distinct. They are considered as representatives of three biotypes, which may correspond to Harrington's (1945) nos. 2, 3 and 4.

Figure 1 shows also some of the properties of antibiosis of the varieties Pride and Onward. On Perfection, a susceptible variety, the rate of reproduction was linear in the three biotypes. On Pride, all the biotypes declined in rate of reproduction on the fifth day, this being more pronounced in R2 and R3. On Onward, the decline began after the second day. The antibiosis observed is therefore a mechanism that interfered with the production of nymphs after 2, 3 or 5 days depending on the biotypes and the host plants tested. The rate of reproduction was not immediately affected because of carry-over effects of adequate feeding of the test females: the curves show that during the first 2 days biotype R1 reproduced at the same rate on the varieties: 46.5 nymphs on Perfection, 47.5 on Pride and 47.7 on Onward. This carryover effect was also present in the smaller biotypes but was not so clear-cut.

The decline in rate of reproduction was accompanied by overactivity in the females, suggesting that they did

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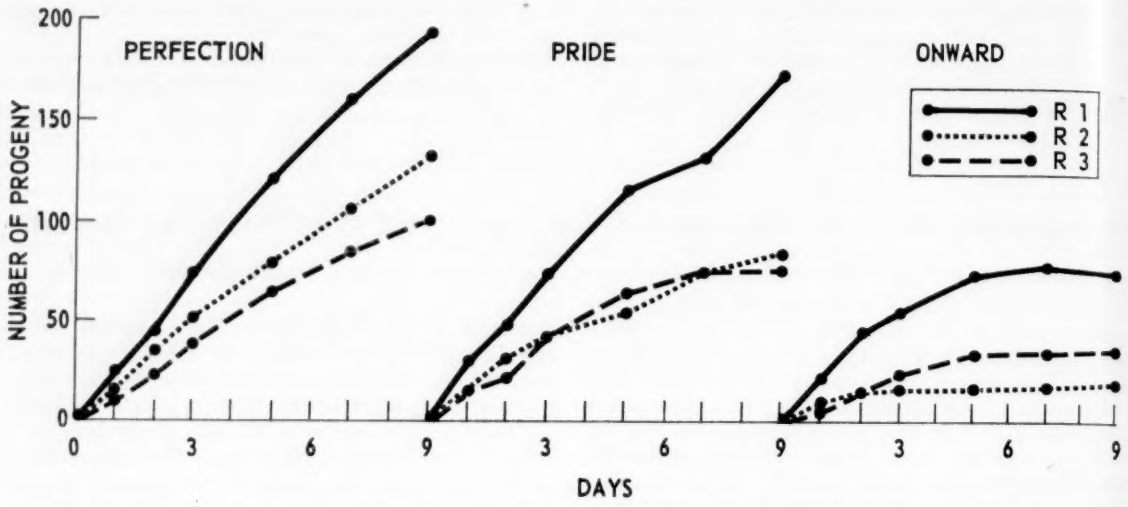

FIG. 1.—Cumulative numbers of progeny in three lines (R1, R2 and R3) of the pea aphid reared on three varieties of peas during 9 days at 69° F. Each curve represents the mean progeny of four replications of three apterous females.

not take or receive enough food from the plant to fulfill the requirements for normal reproduction. The overactive females deposited their progeny in a dispersed pattern on Pride and Onward but in aggregations on Perfection.

The nymphs born on the resistant varieties were not overactive and ended up with a reduced adult weight, indicating that they had been underfed. The feeding behavior of the nymphs was therefore different from that of the adult in parturition.

The relation of biotypes, or strains, of insects to resistance was well summarized by Painter (1951): there are situations when it may be necessary to look further than specific or subspecific differences for insect factors that influence the expression or the permanence of resistance. This is particularly true in the case of aphids. Harrington (1943, 45) reported the existence of five races in the parthenogenetic forms of the pea aphid. The reactions of 31 lines reared on three varieties of peas (Perfection, Pride and Onward), could be grouped in five classes or races. There was a close relationship between size and virulence, indicating that these two characters were quantitatively inherited and that the difference between virulent and nonvirulent aphids might be due to variation in the number of factors for size or vigor in the different genotypes. Cartier & Painter (1956) separated two biotypes of the corn leaf aphid, *Rhopalosiphum maïdis* (Fitch), on resistant and susceptible sorghums. The biotypes differed in fecundity, weight of adults, mortality, production of winged forms, restlessness and aggregation.

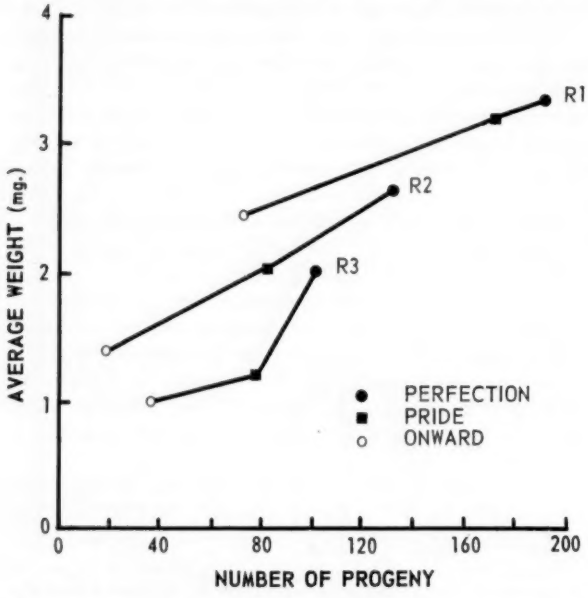

FIG. 2.—Mean weights of females produced and mean numbers of progeny from three females in three lines (R1, R2 and R3) of the pea aphid reared on three varieties of peas during 9 days at 69° F.

The discovery of three biotypes in the pea aphid species led to the belief that several other strains may exist in Quebec. However, the mechanisms of resistance in varieties of peas may not be entirely dependent on the plant since the feeding behavior of different biotypes can alter considerably the measurement of resistance. Consequently, biotype R1 was adopted for a standard culture in the studies on account of its superior size and virulence. The use of biotypes in further research may prove to be of great help in investigating other aspects of aphid resistance.

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Distribution of the First-Generation Egg Masses of the European Corn Borer in Corn Fields[1]

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Abstract

Observations on the distribution of the first-generation egg masses in 20 fields of field corn in Waseca, Minnesota, during 10 seasons, 1948 to 1957, show the following points: 1. The egg populations varied from negligibly low levels of less than 10 egg masses per 100 plants to a high of 380 per 100 plants. 2. The distribution of the egg masses is essentially random, but shows a tendency towards contagiosity. 3. This particular pattern of distribution is followed throughout the entire period of egg deposition in a given field. 4. The frequency index, *i.e.*, the per cent of plants with one or more egg masses, is to an extent directly proportional to the highest egg mass count, *i.e.*, the highest number of egg masses found alive on any one day.

On the basis of these observations, it is suggested that the frequency index may be used as an index for estimation of the density of egg populations. Sixty per cent of plants receiving egg masses will be equivalent to the critical egg mass count of 50. It is believed that the use of the frequency index method involves less work than making an egg mass count.

The distribution of organisms has been classified into three general types: random, contagious and even. These types may be determined by the Poisson series. On the basis of the Poisson series, the expected number of samples containing various numbers of individuals can be calculated for a given mean density of the population. If the observed values fit the expected values, the population concerned will be considered to show a random distribution. Otherwise the distribution will be either contagious or even. If there were relatively more samples with large numbers of individuals in the observed series than in the expected series, the distribution is towards contagious. The reverse will suggest an even distribution.

Even distributions are very rare and are known only in the plant world. Animals in general show contagious distribution because, first, the habitat is usually heterogeneous and secondly, the individuals of a population have usually dispersed from a center of concentration. Random distribution, on the other hand, may result only if the population shows the following characteristics, (1) the individuals did not come from a center of concentration, (2) the individuals exhibit neither amity nor competition, and (3) the habitat is rather homogeneous. Thus random distribution is considered as exceptional and is known in very few instances (Cole 1946, Dice 1948). Among these are the cryptozoic spider, *Scytonotus granulatus*, on the forest floor (Cole 1946), the confused flour beetle, *Tribolium confusum* Duv., in a flour medium (Park 1933), and according to Beall (1940), the egg masses of the European corn borer, *Pyrausta nubilalis* (Hbn.), in a corn field. Walker (1942), however, showed that the distribution of eggs of the corn earworm, *Heliothis zea* (Boddie), is essentially random but shows a tendency towards contagiosity. This particular type of distribution, namely, random with a tendency towards contagiosity, may be more common than suggested by the limited number of records. In fact, Cole (1946a) believed that even the populations which have been demonstrated to have a random distribution may show some degree of contagiosity if the size of the sample is increased.

Beall's (1940) analysis on the distribution of the egg masses of the European corn borer was on the basis of only three sets of records all of which showed rather low population densities. A re-examination of this aspect of the ecology of this insect is needed not only from a viewpoint of population dynamics, but also for its practical implications. The present paper is an attempt to re-examine the problem on the basis of more extensive data.

During the course of studies on the population ecology of the European corn borer in south central Minnesota from 1948 to 1957, detailed observations have been made on the egg populations in a total of 20 fields of hybrid corn. Certain aspects of these observations have been published earlier (Chiang & Hodson 1952). The data accumulated also offer an opportunity to analyze the pattern of distribution of egg masses in corn fields.

Material and Methods.—In most years the fields used were ordinary farm plantings of hybrid corn of the varieties commonly grown in the area. With the exception of some of the fields used in 1950, the fields were planted during the general planting period for the locality and the particular seasons. Varied numbers of plants distributed throughout the fields were selected at random and marked at the beginning of the season. They were carefully checked about twice weekly for egg masses. The masses found each time were marked to avoid confusion when new masses were later deposited on the same plant. In the present discussion only the distribution of the first generation is treated.

This procedure yielded information on the number of egg masses, and also number of eggs, received by each of the marked plants during each half-week period, and also during the entire period of oviposition. Thus it was possible to compute for each field the mean number of egg masses, the highest number of live egg masses found on any one day, *i.e.* the highest egg mass count, and the frequency distribution of plants with different numbers of egg masses.

Based upon the mean density the expected frequency distribution according to the Poisson series was also calculated (Snedecor 1948).

Results.—The results of field observations and the analyses of field data are presented under three categories:

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[3] The work was carried out on the Southern Experiment Station, Waseca, Minnesota. Assistance of Mr. R. E. Hodgson, Superintendent, is greatly appreciated.

Table 1.—Egg populations of corn borer in 20 different fields of corn.

Field No.	Total No. of Egg Masses Deposited/100 Plants	Total No. of Eggs Deposited/100 Plants	Highest Daily Egg Mass Count/100 Plants	Frequency Index (Per Cent Plants with One or More Egg Mass)
4	380	5,798	177	92.5
5	212.5	3,503	90	77.5
8	207	2,953	83	75.0
2	174.2	2,395	87	61.1
12	120.8	1,754	47	59.2
9	65	873	32	50.0
13	64.8	1,057	25	44.0
3	57.2	902	27	41.1
6	32.5	533	12	22.5
16	28	448	14	26.0
1	26	380	12	21.3
19	22	376	9	10.8
17	19	204	6	12.0
7	15	175	15	15.0
10	12.5	120	10	12.0
14	11	151	3	11.0
15	4	75	1	4.0
20	3	28	2	3.0
11	0	0	0	0
18	0	0	0	0

1. The total number of egg masses and eggs deposited during the entire period of the first-generation oviposition, the highest number of egg masses on any one day, and the number of plants receiving any masses at all. These records are presented for all the 20 fields studied (table 1).

2. The observed numbers of plants receiving various numbers of egg masses during the entire period of first-generation oviposition are compared with the expected numbers according to the Poisson series. These results are too bulky to be presented in whole. Thus results of only 12 fields are presented. Nevertheless these fields cover the main range of egg population densities (table 2).

3. The observed numbers of plants receiving various numbers of egg masses up to various dates during the period of oviposition are compared with the expected numbers according to the Poisson series. Records of only one field which received the highest egg population are given. They are sufficient to show the trend (table 3).

DISCUSSION ON DISTRIBUTION OF EGG MASSES.—The 20 fields observed during the 10-year period showed a wide range of egg populations, from a high of 380 egg masses to 0 per 100 plants (table 1). These variations were due to the fluctuation in the natural borer population in the area and the different attractiveness of the

Table 2.—Per cent plants bearing different numbers of egg masses during the entire period of oviposition in 12 fields.[a]

Field No.	Per Cent Plants Bearing Different Numbers of Egg Masses													Frequency Index
	0	1	2	3	4	5	6	7	8	9	10	11	14	
4	7.5 (2.3)	10.0 (8.6)	20.0 (16.2)	22.5 (22.4)	7.5 (21.2)	12.5 (16.1)	10.0 (10.2)	(5.6)	5.0 (2.5)	(1.1)		2.5	2.5	92.5 (97.7)
5	22.5 (12.0)	20.0 (25.4)	22.5 (27.0)	10.0 (19.1)	15.0 (10.1)	7.5 (4.3)	(1.6)	2.5 (0.5)	(0.1)					77.5 (88.0)
8	25.0 (12.6)	17.5 (26.0)	20.0 (27.0)	22.0 (18.6)	7.5 (9.7)	5.0 (4.0)	2.5 (1.4)	2.5 (0.4)	(0.1)					75.0 (87.4)
2	38.9 (17.5)	15.6 (30.2)	23.4 (26.5)	6.7 (15.4)	4.4 (6.7)	3.3 (2.3)	4.4 (0.7)	1.1 (0.1)	1.1		1.1			61.1 (82.5)
12	40.8 (29.9)	28.7 (26.1)	17.2 (21.8)	6.9 (8.8)	4.2 (2.6)	1.9 (0.8)		0.4	0.9					59.2 (70.1)
9	50.0 (42.7)	42.5 (36.2)	5.0 (15.4)	2.5 (4.3)	(0.9)	(0.5)								50.0 (57.3)
13	56.0 (52.2)	28.2 (33.8)	12.5 (10.8)	2.3 (2.4)	1.0 (0.8)									44.0 (47.8)
3	58.9 (57.2)	31.2 (32.7)	5.5 (9.3)	3.3 (1.7)	(0.1)	1.1								41.1 (42.8)
6	77.5 (72.2)	15.0 (23.5)	5.0 (3.8)	2.5 (0.5)										22.5 (27.8)
1	78.7 (74.1)	17.0 (22.2)	3.7 (3.3)	0.5 (0.4)										21.3 (25.9)
7	85.0 (86.0)	15.0 (12.9)	(1.1)											15.0 (14.0)
10	87.5 (88.3)	12.5 (11.0)	(0.7)											12.5 (11.7)

[a] The expected per cent according to Poisson series is given in parentheses.

Table 3.—Per cent plants bearing different numbers of egg masses up to various days during the period of oviposition in Field No. 4.[a]

Dates	No. Egg Masses/100 Plants	Per Cent Plants Bearing Different Numbers of Egg Masses (0–14)													Frequency Index
		0	1	2	3	4	5	6	7	8	9	10	12	14	
June 21	7.5	92.5 (92.5)	7.0 (7.5)	0.5											7.5 (7.5)
26	80.0	55.0 (44.9)	27.5 (36.0)	10.0 (14.4)	2.5 (3.8)	2.5 (0.9)		2.5							45.0 (55.1)
29	165.0	27.5 (19.2)	30.0 (31.7)	20.0 (26.2)	7.5 (13.9)	10.0 (5.7)	2.5 (1.9)	(0.5)		2.5					72.5 (80.8)
July 4	290.0	15.0 (5.5)	20.0 (15.9)	20.0 (23.1)	17.5 (22.4)	5.0 (16.2)	7.5 (9.4)	5.0 (4.4)	5.0 (1.8)	(0.7)	(0.2)	2.5	2.5		85.0 (94.5)
10	327.0	7.5 (3.8)	20.0 (12.4)	17.5 (20.3)	25.0 (22.1)	5.0 (18.0)	10.0 (11.8)	5.0 (6.4)	2.5 (3.0)	2.5 (1.2)	(0.4)	(0.1)	5.0		92.5 (96.2)
17	365.0	7.5 (2.5)	12.5 (9.5)	22.5 (17.4)	20.0 (21.2)	5.0 (19.3)	12.5 (14.1)	10.0 (8.6)	(4.5)	5.0 (2.2)	(0.8)	(0.3)	2.5	2.5	92.5 (97.4)
25	380.0	7.5 (2.3)	10.0 (8.6)	20.0 (16.2)	22.5 (22.4)	7.5 (21.2)	12.5 (16.1)	10.0 (10.2)	(5.6)	5.0 (2.6)	(1.1)	(0.4)	2.5	2.5	92.5 (97.7)

[a] The expected per cent according to Poisson series is given in parentheses.

plants in the fields (Chiang & Hodson 1952).

The main attention at this time is to be directed to table 2. The fields are arranged according to the density of egg masses. As the mean density increases, the per cent plants with no egg masses decreases, and there is an increasing per cent of plants bearing greater numbers of egg masses. For example, in field No. 10 which has a mean density of 12.5, there was no plant having more than one mass. In field No. 13 which has a mean density of 64.8, some plants had as many as 4 masses. But in field No. 4 which had a mean density of 380, as many as 14 masses were found on one plant. The distribution can perhaps be more clearly seen in the graphic form. To avoid crowding in the graph, only six fields which cover the main range of densities are presented in figure 1. The curves conform with those of a random distribution in a general way.

Further analysis can be made by comparing the observed numbers of plants which have any egg masses at all in the 12 fields, and the corresponding numbers expected according to the Poisson series (right-hand column in table 2). These data are also presented graphically in figure 2. It may be noted that the observed values are all smaller than the expected values. This means that there were more plants with no egg masses than expected on the basis of a Poisson distribution. It has been demonstrated by various workers that plants of a more advanced stage of growth receive more egg masses than younger plants. Spotted examinations of the records show that the taller plants in a given field also received more egg masses than the shorter plants in the same field. This has resulted in a concentration of egg masses which will account for the slight tendency towards contagiosity in the distribution of egg masses in a field. However, the differential attractiveness of the plants is not great. Hence the overall distribution of the egg masses is essentially random.

The distribution of the corn borer egg masses is comparable to that of the eggs of corn earworm as shown by Walker (1942). It is, however, not apparent in Beall's analysis which involves only three fields of low egg populations. The fields in the present study which have low

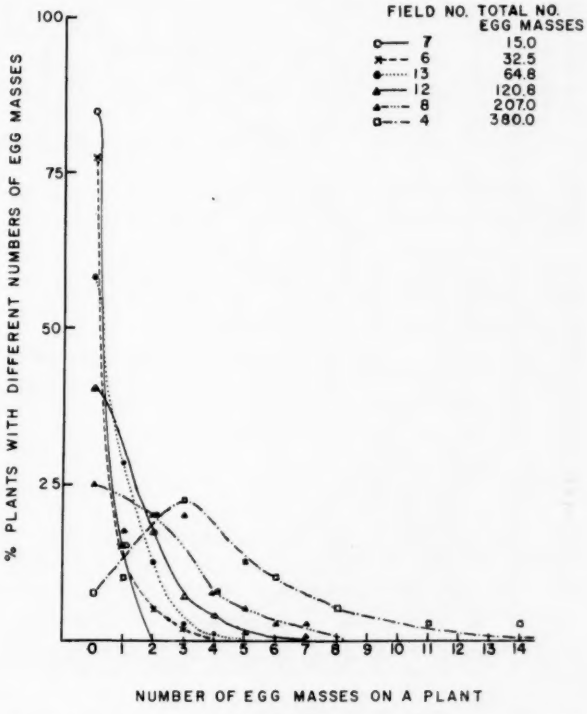

FIG. 1.—Per cent of plants with different numbers of egg masses per plant in six fields with egg populations of different mean densities.

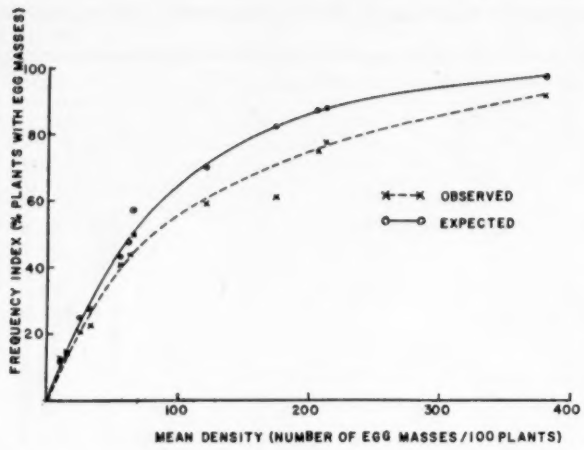

FIG. 2.—Comparison of the observed and expected frequency index in fields with egg populations of different densities.

populations also show a more typical random distribution (see figure 2).

The next question is whether the distribution of the egg masses deposited at different times during the oviposition period follows the same pattern. Records taken in field No. 4 which has the highest egg population were analyzed. The per cent plants with various numbers of egg masses cumulated to different days are shown in table 3, and also in figure 3. It is clear that the curves are very similar to those in figure 1, and are also essentially random. This fact suggests that the same pattern of distribution of egg masses among the plants in a given field was followed throughout the entire period of egg deposition.

DISCUSSION ON FREQUENCY INDEX.—In the practice of controlling the first-generation corn borer an egg mass count of 50 is sometimes considered as the critical level of infestation above which control is recommended. This count represents the highest number of egg masses found alive on any one day during the entire period of oviposition. The use of egg mass count is recommended because it can be more conveniently checked than the total egg masses. Chiang & Hodson (1952) have shown that the egg mass count is directly proportional to the total number of egg masses, and established the biological soundness of using egg mass count as a basis for estimating the level of egg population. The results presented in this paper suggest that the level of egg population can be established on another basis, namely, the frequency index.

Frequency index in general is the per cent of samples having one or more individuals (Dice 1948), and in the present study may be expressed as the per cent plants with one or more egg masses. Table 1 lists the egg mass count and the frequency index for the different fields. These two sets of figures are again graphically presented in figure 4. With the exception of one record, all points fall in a smooth curve. This curve describes the manner in which the frequency index is proportional to the egg mass count. From a mathematical point of view, this relationship between the egg mass count and the frequency index is to be expected since the distribution of the egg masses was essentially random, and since the egg mass count is directly proportional to the total number of egg masses. The similarity of the curves in figure 2 and figure 4 also confirms this view.

As mentioned earlier, the egg mass count of 50 is considered as the critical level. On the curve in figure 4, this egg mass count corresponds to a frequency index of 60%. In other words, a frequency index of 60% indicated the

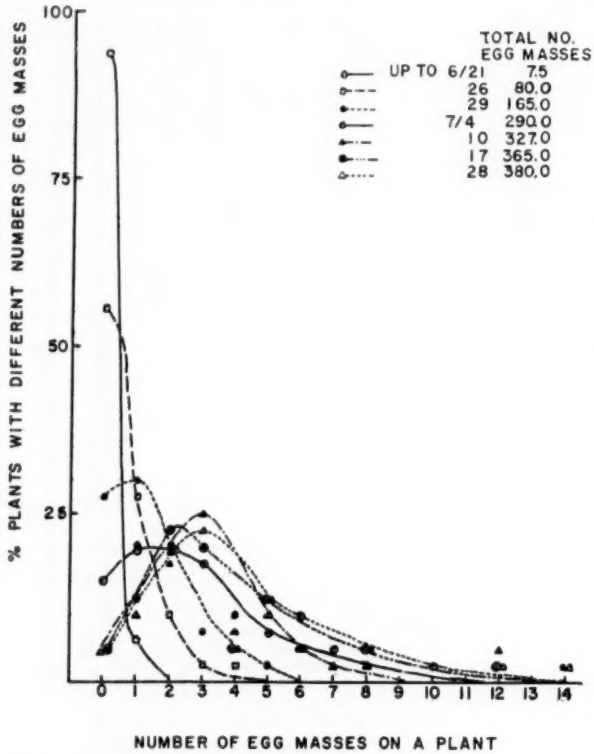

FIG. 3.—Per cent of plants with different numbers of egg masses per plant in field No. 4, up to different dates during the oviposition period.

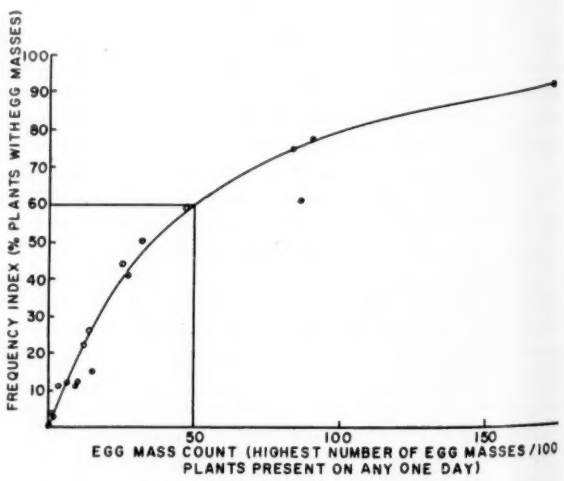

FIG. 4.—Relationship between per cent of plants with egg mass or egg masses, and the highest "egg mass count."

same population density as indicated by an egg mass count of 50.

This curve also shows a feature common to all animal populations, namely, at high densities, the index invariably approaches 100%. In other words, the frequency index is insensitive as an indication of population density for populations of high densities. This feature however is of only academic significance since our interest is in populations of a rather low level. The level of the egg mass count of 50 is well within the sensitive range of frequency index.

In the use of frequency index as a means of estimating the abundance of egg masses, a grower may stake out 100 or 50 plants in the field. The first checking for egg masses will be made on all the plants. Those showing the presence of egg masses will be marked. The second checking will be made only on the plants which had no egg masses during the first checking. The new plants which showed egg masses will be marked as before. Subsequent checking will be made until 60 or 30 plants are marked. This means that a frequency index of 60 has been reached. This procedure will require less time than checking the egg mass count, because first, fewer and fewer plants will be checked during the subsequent checkings. Secondly, the checking on a given plant is to determine only the presence or absence of egg masses, and not the number of egg masses present. Thus the checking of a given plant may be stopped as soon as a mass is found on the plant.

Another significant point should also be mentioned. Figure 3 shows that the cumulation of egg masses during the oviposition period in a given field follows the same pattern as the final egg mass abundance in fields of different population levels. Thus the proportionality between egg mass count and frequency index will be the same at all times during the period of oviposition. In other words, whenever 60% of the plants showed one or more egg masses each, the egg mass count would have just reached 50, no matter on what day during the oviposition period this happened.

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Survival of Insect Eggs After Stratospheric Flights on Jet Aircraft[1]

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ABSTRACT

Studies were made to determine whether insect eggs on the exterior surfaces of jet aircraft survive flights in the stratosphere. The first flight was at 40,000 feet for 8 minutes at Mach 0.82 (465 knots true air speed) and an air temperature of −57° C.; the second at 45,000 to 50,000 feet for 30 minutes with 12 minutes above Mach one and an air temperature of −60° C. Complete mortality of eggs of the yellow-striped armyworm (*Prodenia ornithogalli* Guen.), a representative species of Phalaenidae, the only family reported as laying eggs on aircraft, was obtained in both tests. There was, however, a normal hatch of the cold-resistant eggs of the eastern tent caterpillar (*Malacosoma americanum* (F.)) under these conditions.

The finding of viable insect eggs on the exterior surfaces of propeller-driven aircraft caused concern that agricultural pests might be transported from infested to non-infested areas of the world in this manner. At the Miami, Fla., quarantine station Porter & Hughes (1950) examined the exterior of 9,055 planes for insect egg masses and found them on 78. The number per plane ranged from 1 to 1000. The viable eggs were permitted to hatch and were identified as belonging to the moth family Phalaenidae, which includes some of our most serious agricultural pests. Laird (1952) examined 342 aircraft that arrived in Auckland from Fiji, Norfolk Island, and Australia, and collected moth egg masses from the exterior surfaces of 32. Senior-White & Kirkpatrick (1949) and Dumbleton (1950) have reported similar discoveries.

The advent of jet aircraft with high-speed flights in the stratosphere raised the question as to whether the egg masses would be removed by air turbulence or killed by the low temperatures at high altitudes. The answer was sought by exposing egg masses on the outer surface and other locations of U. S. Navy jet aircraft during routine missions at the Naval Air Test Center, Patuxent River, Md.[2]

The yellow-striped armyworm was selected as a test insect because it is typical of the Phalaenidae. Laboratory-reared specimens readily layed egg masses on the aluminum side of aluminum foil-paper laminate when the foil was used as a lining to battery-jar cages.

The eastern tent caterpillar was also used to give infor-

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[2] Appreciation is expressed to Lt. Commander Frank H. Austin, MC, Head, Aeromedical Branch, Service Test, Naval Air Test Center, Patuxent River, Md., for advice and assistance and for flying the first mission, and to Commander Jack L. Morrissey for flying the second mission.

Table 1.—Flight profiles of test aircraft.

ALTITUDE (1000 FEET)	TEMPERATURE (° C.)	TIME (MINUTES)	SPEED (KNOTS TRUE AIR SPEED OR MACH NUMBER)
	F9F-8 Cougar		
0 to 16	+12 to −14	5	300
16 to 5	−14 to + 5	10	400
5	+ 5	70	200
5 to 40	+ 5 to −57	10	300
40	−57	8	465 (MACH 0.82)
40 to 0	−57 to +12	5	400
	F8U-1 Crusader		
0 to 45	+14 to −60	8	MACH 0.9
45 to 50	−60	30	MACH 0.9 to 1.25 0.2 hr. above MACH one (1.0)
50 to 0	−60 to +14	22	MACH 0.9

mation on survival of a cold-resistant insect. The egg masses were collected from cherry trees on the day prior to tests (Nov. 6 and 20, 1957).

Studies on the survival of these egg masses were made in two flights of jet aircraft into the stratosphere. The first test was made during a flight of an F9F-8 Cougar; the second during that of an F8U-1 Crusader aircraft. The flight profiles are given in table 1.

In the first flight 1- to 2-inch squares of aluminum foil each containing one to three armyworm egg masses and twigs with tent caterpillar egg masses attached were secured with Scotch tape (fig. 1) at various locations on the upper and under surfaces of the wings. A kraft paper bag containing both species was also placed in the radar compartment in the nose cone.

When the aircraft landed, two of the four twigs with tent caterpillar eggs and one of the five aluminum squares containing armyworm egg masses were missing. Microscopic examination showed little or no damage to the recovered egg masses. The armyworm eggs were kept under observation in a constant-temperature room (25° C.) until they hatched in 2 to 3 days. The tent caterpillar eggs were placed in a refrigerator at −10° C. for about a month and then for another month at 2° to break the diapause. They were then tied to branches of cherry trees in a greenhouse maintained at 24°–27° C. until they hatched, about 7 days later.

In the second flight the eggs were exposed in a similar manner except that they were placed in screen cages in the ammunition compartment instead of in the nose cone. All egg masses were intact when the aircraft landed. A post-flight photomicrograph of an armyworm egg mass on the under-wing surface is shown in figure 2.

The per cent survival of the egg masses in both flights is given in table 2. In both flights all the armyworm egg masses on the outside of the aircraft were killed. This indicates that the habit of Phalaenidae of laying eggs on the exterior of aircraft does not present a quarantine problem. A 3% hatch of armyworm egg masses in the

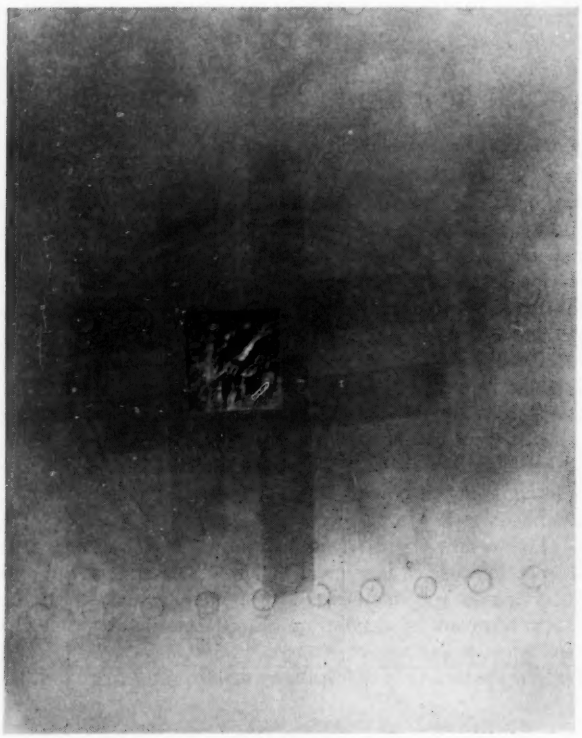

FIG. 1.—Armyworm egg masses on aluminum foil secured to the side of an F-8U1 Crusader with Scotch tape.

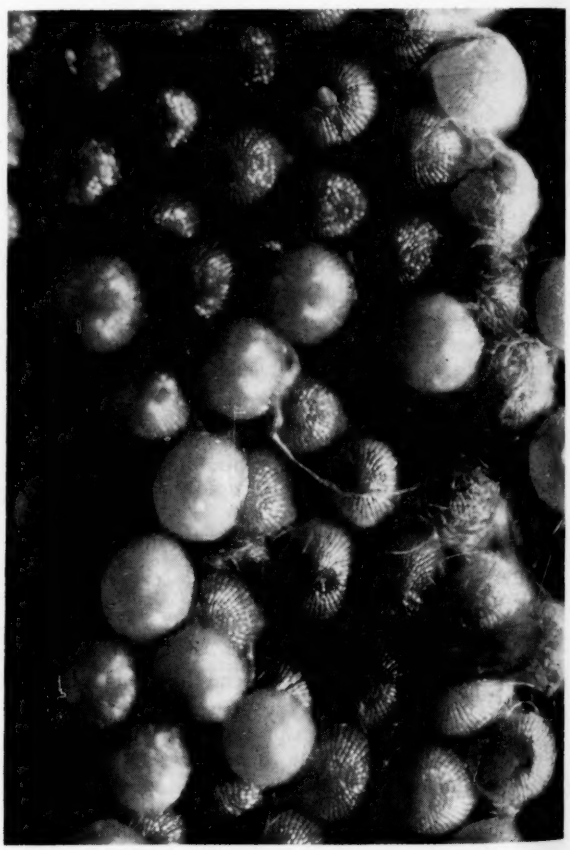

FIG. 2.—An armyworm egg mass after a 30-minute flight at 45,000 to 50,000 feet (−60° C.) with speeds as high as Mach 1.25.

Table 2.—**Survival of egg masses of yellow-striped armyworm and eastern tent caterpillar after stratospheric flights in jet aircraft.**

Insect and Location of Egg Masses	Number of Egg Masses	Per Cent Survival
Flight 1. F9F-8 (Cougar)		
Yellow-striped armyworm:		
Outer center, upper surface of wing	6	0
Inner center, upper surface of wing	1	0
Center, under surface of wing	1	0
Radar compartment (nose cone)	6	3
Upper and under surfaces of wing (check)[a]	6	97
Eastern tent caterpillar:		
Wing-fold indentation, rear of upper wing	1	
Upper surface, where wing joins fuselage	2	
Radar compartment in nose cone	14	Normal hatch
Upper and under surfaces of wing (check)[a]	5	
Flight 2. F8U-1 (Crusader)		
Yellow-striped armyworm:		
Near wing tip, upper surface of wing	1	0
Center, upper surface of wing	1	0
Inner center, upper surface of wing	2	0
Center, under surface of wing	4	0
Each side of fuselage, ½ from front	2	0
Ammunition compartment	3	5
Landing gear door	1	0
Speed brake	1	0
On steel door facing sun (check)[b]	10	31
Eastern tent caterpillar:		
In groove between upper surface of wing and fuselage (each side of aircraft)	2	
Landing gear door	1	
Speed brake	1	Normal hatch
Ammunition compartment	1	
On steel door facing sun (check)[b]	6	

[a] AD-7 that remained on ground.
[b] Steel door of hangar.

nose cone of the F9F-8 Cougar was due to the heat given off by electronic equipment. The 5% hatch in the ammunition compartment of the F8U-1 Crusader can be explained by some heating due to the presence of generators and heat exchangers, which are driven by air from the compressor section of the engine. This is in agreement with earlier work by Sullivan *et al.* (1958), who found that insects may be protected from cold by heat given off by electronic and other equipment.

The tent caterpillar eggs on the exterior of an aircraft were able to survive a flight at 45,000 to 50,000 feet for 30 minutes with 12 minutes above Mach one and an air temperature of −60° C. Possible explanations for this remarkable resistance are: (1) the tent caterpillar overwinters in the egg stage throughout eastern Canada, and (2) the wing surfaces may have been warmer than the surrounding air owing to heat from the sun or friction. It is suggested that the tent caterpillar is an excellent insect for studying the effects of high altitudes on insects.

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The Toxicity of Several Insecticides to the Eggs and Larvae of the Pink Bollworm[1]

J. R. Brazzel[2] and J. C. Gaines[3]

Abstract

Toxicity tests were conducted at College Station, Texas during 1956 to determine the effect of several insecticides on eggs and larvae of the pink bollworm, *Pectinophora gossypiella* (Saund.). Guthion and parathion were effective as ovicides when applied directly to eggs and when sprayed on infested plants. Significant reductions in pink bollworm larvae were obtained with all insecticide treatments compared with the check but Guthion and DDT were superior to the other materials.

Chemical control measures for the pink bollworm have been developed within the last 10 years with the discovery that certain of the organic insecticides were effective for its control (Robertson 1948, Ivy *et al.* 1955, Bottger *et al.* 1958). Results obtained by Robertson (1948) indicated that field control was obtained principally by killing the adult. In field cage tests Williams *et al.* (1958) found that Guthion and DDT killed large numbers of adults and also reduced the numbers of eggs laid by the survivors. Since a large percentage of the pink bollworm eggs are deposited in a protected site under the boll calyx (Brazzel & Martin 1957), the question arose as to what effect these insecticides had on eggs and larvae. Smith (1952) reported that the effectiveness of parathion for control of the peach tree borer (*Sanninoidea exitiosa* (Say)) was due in part to ovicidal action. The research reported herein was conducted to determine what degree of control of the eggs and first instar larvae could be expected with insecticides in current use.

Materials and Methods.—*Egg tests.*—Pink bollworm eggs were obtained by placing adults in an oviposition cage provided with a green cotton leaf over a screened opening. The moths oviposited through the screen wire on

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[2] Louisiana Agricultural Experiment Station, now with Texas Agricultural Experiment Station.

[3] Texas Agricultural Experiment Station.

the cotton leaf. The eggs were collected daily and used the same day in tests. Eggs were transferred in groups of 10 from the leaf to a 1-square-centimeter tab of paper. Treatments were made by dropping approximately 25 microliters of a known concentration of the toxicant onto the eggs. The technical grade of the insecticide dissolved in acetone was used and an acetone treated check was maintained in all tests. Each group of 10 eggs was considered one replication and each test was replicated five times. Sufficient tests were conducted with each insecticide to obtain an LD-50. The number of tests conducted and insecticides included were as follows: a. DDT (10), b. toxaphene (6), c. dieldrin (8), d. endrin (8), e. lindane (8), f. heptachlor (6), g. malathion (6), h. parathion (42), i. Guthion (57).

The treated eggs were held for 5 days in vented plastic containers. Mortality counts were made by counting all eggs that did not hatch during this period. Per cent control was obtained by using Abbott's (1925) formula. Respiratory rates were determined by the procedure described by Umbreit *et al.* (1949). Respiratory rates were obtained for eggs treated with DDT, Guthion and an acetone-treated check. A total of 100 eggs in each reaction vessel was used for the determinations.

Larval tests.—A modification of the procedure described by Ivy & Scales (1950) was used. All tests were conducted in a screened greenhouse to prevent natural infestation of the bolls by pink bollworm and other insects. Deltapine-15 cotton was planted in 1-gallon pots and allowed to fruit. White blooms were tagged daily and used when a crop of bolls attained the age range of from 10 to 20 days. Freshly laid pink bollworm eggs were counted out into lots of 5 each. Each boll was infested by placing 5 eggs at each of 2 locations under the calyx. A tip of the calyx was lifted with a probe, eggs were placed under it with a moist camel's-hair brush, and the calyx allowed to drop back over them. The infested plants were treated approximately 3 days later in order that the eggs would hatch within 12 hours after treatment.

The infested plants were treated under a spray chamber with the insecticide sprayed on from above at approximately 60 p.s.i. After spraying, the chamber was left in place for 3 minutes before the treated plant was removed. Insecticides used were the emulsified concentrates available commercially. Sprays were applied at the rate of 7½ gallons of mixture per acre. Rates of technical toxicant per acre were those currently being used in insect control programs and are given in table 1.

The infested bolls were collected 10 days after treatment and examined with a binocular microscope. Records were made of number of eegs that hatched; number of entrance holes; where the larvae entered the boll, *i.e.*, above or below the calyx; and number of larvae recovered per boll. The tests were replicated six times and the number of bolls used for each insecticide is given in table 1.

RESULTS AND DISCUSSION.—As indicated by the dosage-mortality curves (fig. 1) two organophosphorus insecticides, Guthion and parathion, were much more effective as ovicides than the other insecticides tested. Malathion, also an organophosphorus insecticide, was included but the LD_{50} fell within the range of those for the chlorinated hydrocarbon insecticides. Lindane, endrin and DDT were more effective as ovicides than dieldrin, heptachlor and toxaphene. These results indicate that Guthion and parathion are good ovicides when applied to pink bollworm eggs.

It was observed that apparently complete larval development occurred in the eggs that did not hatch. The embryo apparently developed normally to the point of hatching and then died. This was apparent with all the insecticides used. Smith (1952) observed a similar phenomenon with peach tree borer eggs treated with parathion. A comparison of the oxygen uptake of eggs treated with DDT and Guthion with untreated eggs indicated that death of the embryo did not occur until within a few hours of hatching.

Results of the larval toxicity tests are also presented in table 1. It was possible in these tests to obtain data on the ovicidal effect of the insecticides when sprayed on the infested plants in a manner comparable with field applications of insecticides. The percentage of eggs that hatched in Guthion and parathion treatments was 57.0 and 60.9, respectively, compared with 79.6 in the check. These reductions were significant, indicating that some control was obtained with these two materials through ovicidal action. Slight reductions in the percentage of eggs that hatched were evident in all other insecticide treatments but none was significant when compared with the check. These results are similar to those obtained in the tests for ovicidal action already discussed.

The percentage of larvae that entered bolls was based on the numbers of entrance holes found outside the boll

Table 1.—Effectiveness of several insecticide sprays for control of pink bollworm eggs and larvae 1956, College Station, Texas.

Insecticide	Pounds Technical per Acre	No. Bolls Tested	Per Cent Eggs Hatched	Per Cent Larvae Entered Bolls	Per Cent Larvae Entered Above/Below Calyx	Per Cent Larvae Recorded	Per Cent Control of Larvae
DDT	1.5	90	66.1	20.2	44/56	15.1	70.5
Toxaphene	3.0	88	69.1	39.5	62/38	28.6	42.3
Dieldrin	0.3	81	70.5	44.9	71/29	32.8	34.5
Heptachlor	0.4	79	72.2	53.2	69/31	39.1	22.3
Endrin	0.4	75	69.3	39.9	70/30	28.9	41.8
BHC (gamma)	0.3	80	65.8	41.3	71/29	31.0	39.7
Malathion	1.0	77	70.3	37.8	68/32	29.0	44.8
Parathion	0.25	75	60.9	32.1	69/31	22.1	53.1
Guthion	0.25	75	57.0	13.3	36/64	9.1	80.6
Check		72	79.6	68.5	75/25	51.5	

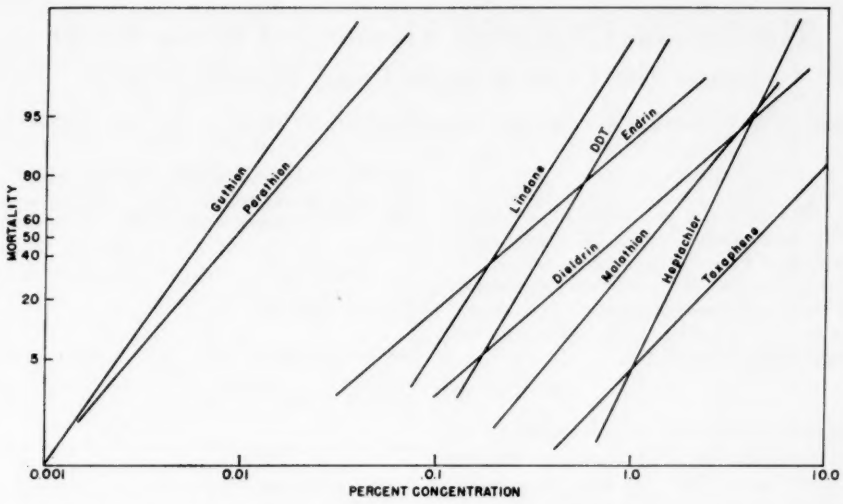

FIG. 1.—Dosage-mortality curves for pink bollworm eggs when treated with acetone solutions of several insecticides.

and the total number of eggs that hatched. Thus, this value is an indication of the effect of the insecticide on first instar larvae and is not complicated with ovicidal effects. All insecticides significantly reduced the number of larvae that entered the bolls compared with the check. However, two insecticides, Guthion and DDT, are clearly outstanding with only 13.3% and 20.2%, respectively, entering compared with 68.5% in the check. These results indicate that some control of first instar larvae was obtained with all treatments and that Guthion and DDT were superior to the other materials.

The pink bollworm adult prefers to oviposit under the calyx of the bolls on older cotton. Thus, the eggs are located in a site that affords some protection from predators and insecticides. Also, larvae enter the boll in this protected site. These factors are often given as reasons why the pink bollworm is so hard to control with chemicals. In this study it was found that, even though all eggs were placed under the calyx, 75% of those that hatched, emerged and entered the cotton boll above the calyx in the check. A higher percentage of larvae entered the bolls above than below the calyx in all treatments except Guthion and DDT. However, more larvae were able to enter the bolls below the calyx than above in the more effective Guthion and DDT treatments. These results indicate that the first instar larvae that were killed by insecticides were those that entered the boll above the calyx. The differences obtained in reduced percentage of larvae that entered the boll above the calyx were significant for Guthion and DDT compared with the check and all other treatments.

The percentage of larvae recovered from the bolls based on number of eggs that hatched and per cent control are given in table 1. Significantly fewer larvae were recovered from all insecticide treatments than from the check. Again, Guthion and DDT gave outstanding control of larvae compared with the other treatments.

The results presented herein indicate that significant kill of pink bollworm eggs was obtained with Guthion and parathion when applied directly to the eggs or sprayed on the infested cotton plant. However, parathion was considerably less effective for control of larvae than either Guthion or DDT. This indicates that, although the ovicidal action of the insecticide probably contributes to the overall control of the pink bollworm, the most effective control was obtained by the action of the insecticide on the first instar larvae that emerged from below the boll calyx and attempted to enter the exposed portion of the boll above the calyx.

Williams *et al.* (1958) reported that insecticide treatments caused mortality in the adult pink bollworm as well as a reduction in the number of eggs deposited on the treated plant by survivors. Therefore, effective chemical control of the pink bollworm results from killing eggs, larvae and adults as well as reducing oviposition of the survivors on treated plants.

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The Residual Action of Thimet and Bayer 19639 against the Cotton Aphid and the Greenbug[1]

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Abstract

Thimet and Bayer 19639 applied at 10, 20, and 30 pounds per acre as a side dressing significantly decreased the numbers of cotton aphids (*Aphis gossypii* Glov.) for 15 months. Seed or furrow treatments with 1 to 3 pounds per acre at time of planting significantly reduced aphid populations for one growing season. Control of greenbugs on oats in rotation with cotton that had received Thimet side-dressings was indicated.

One of the problems confronting entomologists in attempts to control the boll weevil (*Anthonomus grandis* Boh.) with Thimet and Bayer 19639 has been to maintain a sufficiently high level of the toxicant in the plant. In 1956 and 1957 several seed and side-dressing treatments were tested in plots at Florence, S. C., in an effort to accomplish this end. These treatments proved rather ineffective against the boll weevil (Hopkins *et al.* 1958), but some interesting data were obtained on the cotton aphid (*Aphis gossypii* Glov.).

All seed treatments were made with carbon formulations containing 44% of Thimet and 50% of Bayer 19639 furnished by the manufacturers. Furrow and side-dressing treatments were made with granulated formulations of the two materials. In a 1956 test, side-dressings of Thimet granules were put down in plots which had received two different rates of fertilizer to determine if soil phosphate level had any measurable effect on the uptake of the toxicant. Fertilizers with and without phosphorus were used. Nitrogen and potash levels were kept constant. After harvest the stalks were shredded, the soil was disked, and the land left fallow during the winter. Cotton was planted in the same plots in April 1957.

During July and August of both years, after it had been shown that all treatments gave early-season control of aphids, six applications of calcium arsenate at approximately 15 pounds per acre were made to build up late-season aphid populations. In August the aphids on 10 leaves from each plot were counted.

In the 1957 side-dressing test, after the stalks had been shredded and the land disked following harvest, oats were planted on the entire area. In November counts were made of greenbugs (*Toxoptera graminum* (Rond.)) on 10 plants in each plot.

In 1956 all seed and furrow treatments gave significant aphid control (table 1). All seed treatments of Bayer 19639 were superior to the Thimet seed or furrow treatments. The furrow treatments of Thimet gave better control than the seed treatment. This may have been due to greater toxicant dissipation with the seed treatment during the test period whereas the Thimet granules in the furrow may have released the toxicant more slowly thus prolonging its effectiveness.

In the 1956 side-dressing test (table 2) aphid populations were very low in all plots during the early summer; but on July 13 those in the treated plots were significantly lower than those in the checks. Following six applications of calcium arsenate, populations in the checks reached much higher levels than those in the treated plots. There was no indication of greater plant intake or translocation of Thimet in plots receiving fertilizer with or without phosphorus.

In July 1957 obvious differences in aphid populations were observed in plots receiving different rates of insecticides in 1956. The entire field was then dusted six times with calcium arsenate to build up aphid populations to determine if the 1956 treatments with Thimet and Bayer 19639 would protect the plants from the aphids. Counts made on August 19, approximately 15 months after treatment, showed that a high protection was still being afforded by all treatments.

To determine whether the Thimet was actually killing aphids in the treated plots, plant terminals were excised, taken to the laboratory, and known numbers of aphids

Table 1.—Cotton aphids on August 10 in plots receiving Thimet or Bayer 19639 at planting time, April 19, 1956.

Treatment	Pounds of Toxicant per Acre	Average Number of Aphids
Untreated check	—	99.9
Seed treatments:		
Thimet	1	43.9
Bayer 19639	1	4.8
	2	0.3
	3	.3
Furrow treatments:		
Thimet	1	26.1
	2	18.0
L.S.D. at 5% level	—	27.3
at 1% level	—	36.8

Table 2.—Cotton aphids in plots receiving Thimet side-dressings on May 25, 1956.

Fertilizer Under Cotton	Pounds of Thimet per Acre	Average Number of Aphids		
		July 13, 1956	August 10, 1956	August 19, 1957
5-10-5	10	0.06	0.3	16.0
	20	.08	.4	2.3
	30	.02	.2	1.8
	None	.13	151.7	74.4
20-0-20	10	.08	2.2	13.3
	20	.05	.2	12.9
	30	.01	.1	2.9
	None	.28	147.7	133.7
L.S.D. at 5% level	—	.01	36.12	61.6
at 1% level	—	.02	48.47	82.7

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[2] In cooperation with the South Carolina Agricultural Experiment Station.

confined on them. Subsequent observations confirmed that mortality actually occurred.

Results of these tests (tables 3 and 4) verified those obtained in 1956. In the seed and furrow treatments Bayer 19639 was again superior to Thimet. However, there was no significant difference between the seed and furrow treatments of Thimet. Furrow treatments in addition to the side dressings proved to have no advantage over the side dressings alone. A cottonseed-oil formulation containing 50% Thimet was no more effective than the carbon formulation and seriously interfered with germination, presumably because moisture failed to penetrate the seed coat.

Since small grains commonly follow cotton in South Carolina and the greenbug is frequently a serious threat to these crops, the residual effectiveness of Thimet and Bayer 19639 against this pest was tested in such a rotation. Oats were planted in October on the 1957 side-dressing test area and on November 22 greenbug counts were made. Highly significant differences in greenbug populations were found (table 4). The treatments applied to cotton were affording varying degrees of greenbug control on oats. These tests indicate that high rates of Thimet and Bayer 19639 may have a long residual action against some insects in the sandy loam soils common to the coastal plains of South Carolina.

Table 3.—Cotton aphids on August 19 in plots receiving Thimet or Bayer 19639 at planting time, April 11, 1957.

TREATMENT	POUNDS OF TOXICANT PER ACRE	AVERAGE NUMBER OF APHIDS
Untreated check	—	31.5
Seed treatments with carbon formulation:		
Thimet	1	15.0
Bayer 19639	1	6.5
Seed treatments with cottonseed oil formulation:		
Thimet with emulsifier	1	16.8
Thimet without emulsifier	1	11.8
Furrow treatments:		
Thimet	1	27.2
Bayer 19639	1	1.0
	2	0.5
	3	0.03
L.S.D. at 5% level	—	13.3
at 1% level	—	17.8

Table 4.—Cotton aphids on cotton and greenbugs on oats in plots receiving Thimet or Bayer 19639 in 1957.

TREATMENT	POUNDS OF TOXICANT PER ACRE		AVERAGE NUMBER OF	
	Side-dressing[a]	Furrow[b]	Cotton Aphids per Leaf August 19	Greenbugs per Plant Nov. 22
Untreated check	—	—	60.6	13.8
Thimet	10	0	0.8	8.1
	20	0	0	4.4
	10	1	0.3	4.4
	20	1	.1	2.5
Bayer 19639	10	0	.0	2.4
	20	0	.0	0.6
	10	1	.0	1.0
	20	1	.0	0.5
L.S.D. at 5% level	—	—	12.8	3.2
at 1% level	—	—	17.2	4.3

[a] Sidedressed May 24, 1957.
[b] Furrow treatments at planting April 11, 1957.

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Winter Survival and Time of Emergence of Diapausing Pink Bollworms in Central Texas[1]

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ABSTRACT

The winter survival of diapausing larvae of the pink bollworm (*Pectinophora gossypiella* (Saund.)) and time of adult emergence was determined for the period 1953–55. When pink bollworm infested seed cotton was held on the soil surface under screen cages from 65 to 90% of the larvae survived and emerged as adults. The peak of moth emergence coincided with the beginning of cotton fruiting. Mean temperature below 70° F. and 2- to 3-week periods of no rainfall caused lowered adult emergence.

The pink bollworm was not a cotton pest of economic importance in Central Texas until 1954. During October 1953 a moderate infestation was found in green cotton bolls in Burleson county after fall rains had caused uncut cotton stalks to start regrowth and fruiting. Infestations during the normal growing season of 1954 were light. During the latter part of July 1954 the College Station, Texas, area received from 4 to 6 inches of rainfall causing cotton to start vigorous second growth. Many farmers attempted to make a top crop and extended the growing season into October. The late crop of bolls was ideal for

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pink bollworm development and infestations in untreated fields increased rapidly to high levels. These heavy infestations late in the year and poor stalk destruction resulted in large populations of diapausing larvae. Large numbers of these overwintering larvae survived, resulting in heavy early-season infestations of pink bollworms and considerable damage to the cotton crop in this area in 1955.

No information was available on the effects of the environment on the survival of diapausing larvae or the time of emergence of the adults in the Central Texas area. Therefore, hibernation experiments were conducted at College Station during 1953–54 and 1954–55 to obtain such information.

PROCEDURE.—Six pyramid type screen emergence cages were each placed over 5 pounds of pink bollworm infested seed cotton on October 10, 1953. The cotton was spread out uniformly over the 9 square feet of soil surface covered by each cage. An application of chlordane dust was made to the soil in a band around each cage and approximately 6 inches from each cage for control of predatory ants. The pink-bollworm-infested seed cotton used in the experiment was obtained at Port Lavaca, Texas, and contained approximately 100 diapausing pink bollworm larvae per pound. The number of larvae per pound of infested seed cotton was determined by an examination of 5 pounds of the cotton selected at random from that used in the experiment.

The pink bollworm adults that emerged from the cotton were collected in an inverted one-half pint screw top glass jar equipped with a screen cone. The glass collecting jar was screwed into a holder attached to the top of each emergence cage. The screen cone was fitted into the mouth of the collecting jar with the convex portion pointing upward when the jar was fastened in place. There was a hole 5 millimeters in diameter at the top of the cone which permitted the pink bollworm moths to enter the collecting jar. After the moths entered the jar, the screen cone prevented them from returning to the emergence cage. Records were made of the numbers and dates of moth emergence from the cages. The collection jars were removed from the emergence cages and taken to the laboratory where the moths were anesthetized with carbon dioxide, counted and destroyed. During 1954, weekly collections of adults were made from Januray 1 until April 23. Collections thereafter were made every 2 days until August 30.

The number of emergence cages was increased to 12 in 1954–55. Infested seed cotton used in the experiment was obtained in Burleson County, Texas, and contained approximately 193 diapausing pink bollworm larvae per pound, determined in the same manner as described above. The seed cotton was harvested on November 10, 1954, and 5-pound randomly selected samples were placed under each emergence cage on December 2, 1954. Pink bollworm moths that emerged during 1955 were collected and counted at weekly intervals from January 1 through April 30. Daily counts were made from May 1 until October 1.

The emergence cages were located in the vicinity of the Entomology Research Laboratory at College Station, Texas. Temperature and rainfall records were obtained from the weather station located approximately 200 yards from the emergence cages. The daily mean temperatures for the week were averaged to get the weekly mean temperature which was plotted on the last day of each 7-day period. The rainfall was recorded daily in inches of precipitation. The total number of moths that emerged during each 7-day period was plotted on the last day of each period as a percentage of the total number that emerged from March 1 through August 30.

RESULTS.—The number of pink bollworms that survived the winter and time of emergence of adults during the 2-year study are given in table 1. The adult emergence records were begun on January 1 and continued through September 1 in 1954 and through October 1 in 1955. The emergence records presented in figure 1 represent the adults that emerged from March 1 through August 30 of each year.

Table 1.—Winter survival of larvae and time of emergence of adults of the pink bollworm at College Station, 1953–54 and 1954–55.

Year	Number Larvae Caged	Number Adults Recovered	Per Cent Survival	Per Cent Recovered		
				Before June 1	During June	After June 30
1953–54	3,000	1,973	65.8	50.4	38.1	11.5
1954–55	11,592	10,830	93.4	32.9	42.9	24.2

During the 1953–54 period 65.8% of the larvae introduced into the hibernation cages emerged as adults between January 1 and September 1 of 1954. The following year 93.4% emerged during the same period of time. These results indicate that the winter survival of diapausing pink bollworm larvae in the College Station area was high.

During the emergence period in 1954, 50.4% of the moths emerged before June 1 and 38.1% during June. During 1955 only 32.9% emerged before June 1 and 42.9% during June. During both years most of the adult emergence occurred during May and June, 88.0% in 1954 and 77.3% in 1955.

The peak of emergence in 1954 occurred during the week of June 6–13 when 18.8% of the adults emerged. A lesser peak occurred in the last week of May during which 14.9% emerged. In 1955 the peak of emergence occurred in the week of May 31–June 6 with 19.2% of the adults emerging. The results for the two-year study indicated that the maximum rate of emergence of overwintering pink bollworms occurred during the first 2 weeks of June. This period of emergence coincides with heavy fruiting of cotton in the College Station area.

The relationship of temperature, rainfall and emergence of overwintering pink bollworm larvae is shown in figure 1 and indicate that mean temperatures below 70° F. caused an immediate reduction in the numbers of emerging moths. Rainfall had a delayed but very pronounced effect on moth emergence. The data presented in figure 1 show that an increase in moth emergence occurs within 10 to 14 days after one-half inch or more of precipitation. The emergence of moths always began to drop after a period of 2 to 3 weeks without rainfall.

DISCUSSION.—Results of a 2-year study conducted at College Station, Texas, to determine rate of survival of diapausing pink bollworm larvae during the winter and time of emergence of adults indicated that from 65 to over 90% of the larvae survived when held on the soil

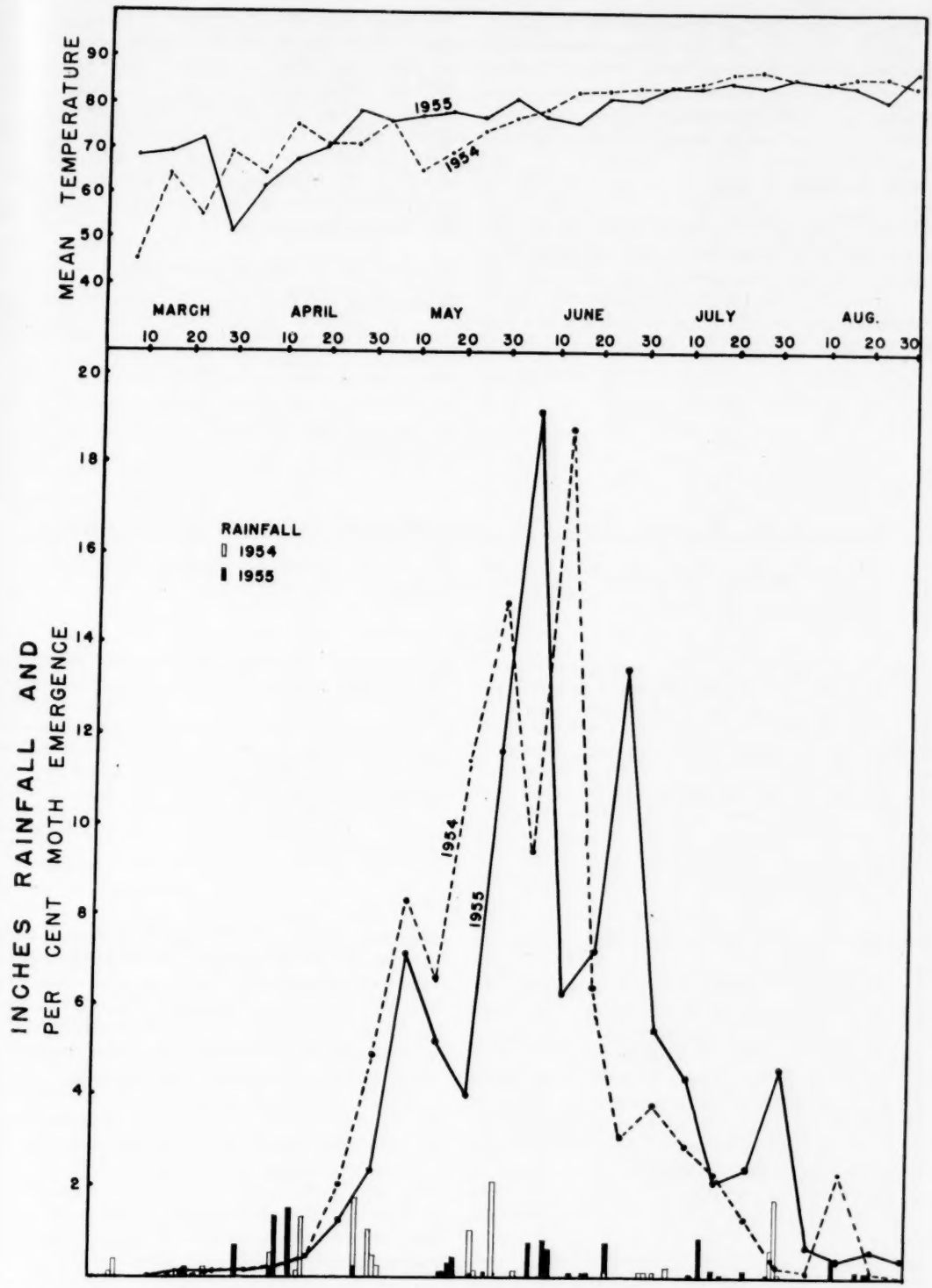

FIG. 1.—Relationship of precipitation and temperature to emergence of adults from diapausing pink bollworm larvae.

surface. From 77 to 83% of the moths emerged during May and June and the peak of emergence for both years occurred during the first 2 weeks of June.

Cotton normally begins to fruit lightly in the College Station area during the period of May 15 to 20. The pink bollworm moth could be expected to live for 10 to 15 days during this period of the year (Fenton & Owen 1953). Allowing 5 days for eggs to hatch because of the cool nights in early May, it is conceivable that some of the offspring of moths emerging as early as May 1 would find cotton

fruit to feed on. Thus, only the moth emergence before May 1 could be considered suicidal. This segment of the emergence is negligible (figure 1). These results indicated that over 90% of the overwintering pink bollworm population emerged at a time when cotton fruit was available for food when the crop residue was left on the soil surface and the land not plowed.

The data presented in figure 1 indicate that rainfall and temperature were important in determining when overwintering larvae would emerge as adults. Mean temperatures below 70° F. appeared to have an immediate delaying effect on adult emergence as illustrated by the results obtained from May 10 to 20 in 1954. The mean temperature dropped to 65° F. and adult emergence was lowered accordingly. The data for the same period during 1955 showed the effect of rainfall on emergence of adults. The mean temperature was between 75° and 80° F. for the period but there was only 0.2 inch of rainfall from April 13 to May 19 which resulted in a reduction of adult emergence from May 10 to 24. The results presented in figure 1 indicate that an increase of adult emergence occurred within 10 to 14 days after ½ inch or more of precipitation. The moth emergence began to decrease when approximately 3 weeks elapsed without rainfall, regardless of high temperatures.

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Insecticide Resistance in Seven Orlando House Fly Colonies[1]

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ABSTRACT

House fly (*Musca domestica* L.) colonies were exposed to various insecticides in studies of the development of resistance. The No. 1 colony, in which experimental development of resistance to DDT was first demonstrated, is in the 270th generation and is maintained in cages coated with DDT; resistance is indicated to be greater than 500-fold. Selection in the LDD colony, now in the 164th generation, is maintained by constant exposure to treated panels. The colony shows 40-fold resistance to lindane, immunity to dieldrin, and 60- to 100-fold resistance to DDT. The C colony, exposed to 0.02% Dipterex in the diet, showed about 10-fold resistance when collected and developed no additional resistance in 98 generations of selection. The Bayer 21/199 colony developed greater than 90-fold resistance to this insecticide in 50 generations, the Grothe colony showed 52-fold resistance to malathion when collected and 100-fold resistance after 20 generations, the Tropical D colony showed 34-fold resistance to Diazinon when collected and 62-fold resistance after 20 generations, and the Tropical P colony showed greater than 18-fold resistance to parathion when collected and no increase in resistance after 20 generations; selection in these colonies was made with contact sprays.

Laboratory colonies of resistant house flies are essential for conducting studies on the basic aspects of resistance and the susceptibility of resistant flies to insecticides. By establishing such colonies it is possible to determine trends of development and loss of resistance in a comparatively short period, for the development of resistance in the field may take several years or more, Laboratory studies with resistant insects also speed up the development of new insecticides and alternate methods of control for use when conventional ones fail. Seven such colonies have been established at the Orlando, Fla., laboratory. Tests to determine their resistance to various insecticides in comparison with a regular normal colony are described in this paper.

TEST PROCEDURES.—Most of the tests were made with contact sprays in a wind tunnel. Female flies 4 or 5 days old were exposed to various concentrations of insecticides in odorless-kerosene solutions. One-fourth milliliter of solution was atomized at a pressure of 1 p.s.i. into the mouth of the tunnel and drawn by a 4 m.p.h. draft through a screen-wire cage containing the flies. After treatment the flies were transferred to screen holding cages and supplied with a 10% sugar-water solution on absorbent-cotton pads. The mortality was recorded after 24 hours. Duplicate tests with 20 female flies were run at each concentration. The LC-50's were then computed from the concentration-mortality data. The ratio of the LC-50 of each colony to that of flies from the normal colony tested at the same time indicated the degree of resistance. The results of these tests are given in table 1.

Bait tests were usually made to follow resistance in the C colony, which originated in localities where baits were the principal method of control. In these tests acetone solutions containing various concentrations of the insecticides were added to dry sugar and the mixtures were allowed to dry. Ten-gram samples were spread over the bottoms of petri dishes; cylindrical screen cages, each containing 20 female flies, were placed on the baits so that the insects could feed *ad libitum* through the screen; and the mortality was determined after 24 hours. Cotton saturated with water was placed on top of the cages after 4 hours. LC-50's were computed and the degree of resistance was determined in the same manner as in the contact-spray tests.

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Residue tests were also used in two of the colonies. Acetone solutions of the insecticides were sprayed on plywood panels at the rate of 100 mg. or 1 gram of active ingredient per square foot. Exposures were made by confining 20 female flies 4 or 5 days old under petri-dish halves on the treated surface for various periods of time. The flies were then transferred to clean holding cages, given food and water, and held for the 24-hour mortality count. All tests were run in duplicate. The degree of resistance was based on the computed LT-50's of the resistant and normal colonies.

No. 1 COLONY, RESISTANT TO DDT.—Lindquist & Wilson (1948) first demonstrated that resistance to DDT could be developed in house flies by selection with this insecticide through 14 successive generations. The colony they established, subsequently designated the Orlando No. 1 colony, was started in January 1946, with flies from the regular laboratory colony, which were exposed to 1 ml. of a kerosene spray containing 1% of DDT in a 100-cubic-foot chamber and then placed in untreated cages. About 10% of the flies survived, and they were used to rear the succeeding generation.

The process was repeated each generation until it became apparent through reduced mortality that an increase in dosage was needed. By the 10th generation the dosage was increased to 1.5 ml., which resulted in a mortality of approximately 65%. The 25th generation was treated with 3 ml, and the 50th generation received 4.5 ml. of 2% DDT. By the 100th generation the mortality was less than 30%, although the flies were sprayed with 25 ml. of 40% DDT and left in the contaminated cages for 24 hours. A group of pupae from the 140th generation were placed in a cage that was completely coated with DDT. Approximately 15% of the females that emerged survived long enough to deposit viable eggs. This colony at the time of writing is in the 270th generation and is maintained by coating the interior of a cage with DDT placing the pupae in the cage, and collecting eggs after 5 days. Very few females are killed.

Resistance to DDT in this colony is so high that no mortality is caused in contact-spray tests with DDT at a concentration of 64%, whereas the LC-50 for regular-colony flies usually ranges from 0.2% to 0.8%. In residue tests with flies of the 245th generation by the use of plywood panels treated with 1 gram of DDT per square foot, no mortality occurred after a constant exposure of 48 hours. As the LT-50 for the regular colony was 5.7 minutes in these tests, the resistance to DDT was indicated to be greater than 500 times.

LDD COLONY, RESISTANT TO LINDANE, DDT, AND DIELDRIN.—The LDD colony was established in July 1951, for the purpose of obtaining a colony of intermediate resistance to chlorinated hydrocarbon compounds for routine testing purposes. This colony was started from the survivors of flies that had been collected at two local dairies and exposed to panels treated with 5 mg. each of lindane, dieldrin, and DDT per square foot. To maintain the resistance at a constant level, two plywood panels treated with 20 mg. of each chemical per square foot were kept in the rearing cage at all times. The old panels were removed and freshly treated panels introduced at weekly intervals. At the time of writing this colony is in the 164th generation.

Table 1.—Susceptibility of female flies of six resistant colonies compared with the regular colony to various insecticides as contact sprays.

INSECTICIDE	GENERATION	LC-50 (%) Resistant Colony	LC-50 (%) Regular Colony	RATIO OF RESISTANCE
		LDD		
Lindane	150th	8.98	0.21	42.76
	155th	10.73	.36	29.80
	160th	18.25	.47	38.82
Dieldrin	140th	>48.00	.12	>400.00
	146th	>48.00	.051	>941.10
	150th	>48.00	.086	>558.10
DDT	150th	22.24	.20	111.20
	155th	23.61	.38	62.19
	160th	27.80	.43	64.65
		C		
Dipterex	82nd	3.54	1.62	2.19
	90th	5.27	.45	11.71
		Bayer 21/199		
Bayer 21/199	24th	0.91	.11	8.27
	40th	1.12	.11	10.18
	45th	>4.00	.073	>54.79
	50th	>8.00	.089	>89.88
	55th	>8.00	.11	>72.72
		Grothe		
Malathion	Parent	47.50	.91	52.19
	5th	27.91	.75	37.21
	10th	22.39	.56	39.98
	15th	13.53	.35	38.65
	20th	>64.00	.72	>88.88
	25th	48.91	.36	135.86
	30th	>64.00	1.32	>48.48
		Tropical D		
Diazinon	Parent	1.20	.035	34.29
	1st	1.40	.036	38.89
	5th	1.14	.051	22.35
	10th	1.94	.066	29.39
	15th	1.03	.021	49.05
	20th	2.56	.041	62.44
		Tropical P		
Parathion	Parent	>.40	.022	>18.18
	1st	.26	.022	11.82
	5th	.26	.033	7.88
	10th	.31	.042	7.38
	15th	.29	.012	24.17
	20th	.52	.028	18.57

The colony shows about 40-fold resistance to lindane, immunity to dieldrin (concentrations of 48% giving little or no mortality), and 60- to 100-fold resistance to DDT.

Residue tests were also run on plywood panels treated with 100 mg. of DDT per square foot with flies of the 98th and 143rd generations of the LDD colony. The degree of resistance indicated by this method of exposure was about 40-fold.

C COLONY, RESISTANT TO DIPTEREX.—The C colony was established in December 1953 to test the possibility of the development of resistance to Dipterex through the use of poisoned baits. The colony was started from flies collected at four barns that had been intensively treated with various organophosphorus baits. Each generation was fed the usual mixture of sugar, powdered milk, and powdered eggs to which 0.02% of Dipterex had been added. For the first five generations the poisoned food had to be removed from the cage after 3 to 5 days to prevent extermination of the colony, but since that time such excessive mortality has not occurred. However, the level of selection remained high, as 68% of the female flies were killed during the preoviposition period in the 96th generation.

When the C colony was first established, the flies were about 10 times as resistant as the regular colony flies to Dipterex in bait tests, but showed no resistance in residue tests. No additional resistance was developed through 98 generations of selection, when the colony was discontinued. At that time the LC-50 in bait tests was 0.02% for

flies of the C colony and 0.002% for regular flies. Resistance in contact spray tests ranged from 2- to 12-fold.

BAYER 21/199 COLONY.—The Bayer 21/199 colony was established to test the possibility of developing resistance to this insecticide by selection with contact sprays. The colony was started in February 1956 from the 101st generation of the LDD colony. Selection was accomplished by exposing 100 to 200 four- to five-day-old flies of undetermined sex to kerosene sprays in the wind tunnel. The concentration of insecticide selected was one that killed approximately 90% of the flies. After treatment the flies were held for 24 hours and the mortality was recorded. The surviving flies were then released in a rearing cage where oviposition medium was available. Eggs were collected and placed in the larval medium for rearing of the subsequent generation. Sprays containing 0.5% of Bayer 21/199 were used in the first 20 generations, 1% in the 21st to 27th, 2% to the 34th, 3% to the 41st, 4% to the 45th, and 10% to the 56th generation, after which the colony was maintained in a cage coated with Bayer 21/199. The colony is now in the 59th generation. Resistance did not exceed 10-fold up to the 40th generation, but thereafter increased rapidly until it could no longer be accurately determined, as the highest concentration of Bayer 21/199 that went into solution killed less than 50% of these flies.

GROTHE COLONY, RESISTANT TO MALATHION.—The establishment of the Grothe colony from a strain of house flies that had become highly resistant to malathion in the field was reported by LaBrecque & Wilson (1957). The strain originated in flies collected in February 1957, in a poultry house at Pine Castle, Fla., where malathion had been used for 2½ years against larvae and adults. Selection to maintain or increase the resistance was accomplished by exposing flies in the wind tunnel, using the technique employed with the Bayer 21/199 colony. The flies of the first 11 generations were sprayed with 32% malathion, which resulted in kills of 50 to 90%. Sprays containing 48% malathion were used from the 12th to the 30th generations, and 64% malathion was used thereafter, which resulted in kills of 80 to 90%. The colony is now in the 35th generation. By the 20th generation resistance was about 100-fold.

TROPICAL D, RESISTANT TO DIAZINON.—The collection of a strain of flies that had developed a high degree of resistance to malathion, Diazinon, parathion, and Dipterex in the field was reported by LaBrecque *et al.* (1958). The Tropical D colony was established from these flies, which were collected from the Tropical Poultry Farm near Miami in June 1957. Selection was accomplished in the same manner as in the Grothe colony. The colony is now in the 22nd generation. Flies of the first five generations were sprayed with 1% Diazinon, and those of later generations with 2%, giving kills of approximately 90%. By the 20th generation resistance was about 60-fold.

TROPICAL P, RESISTANT TO PARATHION.—This colony was established from the same group of flies that were used to start the Tropical D colony, and selection was accomplished in the same manner. The first four generations were sprayed with 0.2% parathion, which gave kills of 70 to 90%; the concentration was raised to 0.4% in the 5th generation, and to 0.8% in the 22nd, which killed approximately 90%. This colony still shows only 18-fold resistance in spite of the high level of selection.

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The Distance into Woods along a Cotton Field at which the Boll Weevil Hibernates[1]

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ABSTRACT

A study of the distance that the boll weevil (*Anthonomus grandis* Boh.) moves into woods adjoining cotton fields revealed that 90% hibernate in the first 180 feet. The most weevils were taken 30 to 45 feet into the woods. High populations were found in trash ½ to 3 inches deep, and more were found in deciduous leaf trash than in pine straw. More weevils were found in slightly moist trash than in exceedingly dry or wet material.

One approach under consideration for control of the boll weevil (*Anthonomus grandis* Boh.) is to apply treatments during the period of hibernation. Both chemicals and diseases are being studied to determine their potential in the control of hibernating weevils. Should either prove effective, the size of the hibernation area to be treated becomes of utmost importance. To determine the area of hibernation in woods bordering cotton fields, studies were initiated at Florence, S.C., in February of 1957 and continued in 1958.

In previous studies at Florence, Bondy (1938) found a large number of overwintering weevils in the first 100 feet of woods. He recommended the destruction or removal of grass, leaves, and litter along the edges of fields and woods as a potential control of these weevils. More re-

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cently Beckham (1957), in 10 samples of woods trash taken at random 50, 150, and 250 feet into the woods, found the largest number at 50 feet in pine straw and leaves. He also found considerable numbers at 150 feet, but only a few at 250 feet. He found few in broomsedge and other grasses adjacent to the cotton field.

METHODS.—During the fall woods-trash examination in 1956 one location in Darlington County had shown an average of 58,104 live weevils per acre. On February 14 and 20, 1957, two swaths (A and B) were taken at that location. A strip 2 yards wide and 270 feet long was staked out for each swath. Individual samples 1 yard long were then taken along the swath, thus providing 90 samples, each 2 square yards in area. These samples consisted of all leaves and litter down to the soil surface. The depth of woods trash; the type of trash—*i.e.*, whether pine needles, deciduous leaves, or a combination of the two; —the number of bushes, trees, vines, or other objects in the area; and the comparative moisture of the trash were recorded. The samples were then run through a standard trash-shaking machine, and the fraction containing the weevils was spread thinly on a heating table for a 10-minute period. The weevils were removed and counted as they emerged.

In 1957 fall woods-trash examinations were made in a location in Florence County that had an average of 11,298 weevils per acre. The following February four swaths 351 feet long (C–F) with 117 samples each were taken in this location. These samples were obtained by the same method and handled in the same manner as those in Darlington County the previous year.

RESULTS.—Data obtained at the Florence laboratory before these studies were made are presented in table 1, and the results of these studies in table 2.

The seasonal weather pattern in South Carolina is such that February-March would probably be the best period in which to control hibernating boll weevils. By this time the colder weather of December and January has usually eliminated the weaker weevils and the spring warming trend has commenced. Therefore, the weevil distribution during this period is of major concern.

A comparison of the two tables shows that the sampling method used in the earlier studies may be somewhat misleading. Populations are variable in the first 15 feet along the edge of the field, and basing the percentages on the total found by the sampling method of the earlier studies places excessive emphasis on the population in that area. This variability is not limited to the edge of the field. In 1957 swaths A and B were taken within a few yards of each other, and swaths C, D, E, and F were also taken within a few yards and parallel to one another. The earlier sampling method, although it does show variability, may not present the true picture.

Table 1.—Accumulative per cent of boll weevils hibernating at various distances in the woods near Florence, S. C.[a]

DISTANCE (FEET)	1938	1953 Fall	1953 Spring	1956
Edge	47.4	38.8	86.8	37.2
50	78.5	64.0	—	67.4
100	87.1	84.4	97.7	77.9
150	100	—	—	91.8
200	—	90.5	100	100
300	—	98.0	—	—
400	—	100	—	—

[a] Results of previous studies.

Table 2.—Per cent of boll weevils hibernating at various distances into the woods in Darlington and Florence Counties, S. C., 1957–58.

DISTANCE (FEET)	DARLINGTON COUNTY A	DARLINGTON COUNTY B	FLORENCE COUNTY C	FLORENCE COUNTY D	FLORENCE COUNTY E	FLORENCE COUNTY F	AVERAGE	ACCUMULATIVE AVERAGE
0– 15	4.8	8.0	3.9	9.0	15.3	22.2	7.4	7.4
16– 30	7.7	11.8	6.9	9.0	10.2	33.3	9.6	17.0
31– 45	9.1	27.7	26.5	16.9	18.6	11.1	19.7	36.7
46– 60	6.2	8.4	14.7	13.5	11.9	0	9.5	46.2
61– 75	6.2	9.2	8.8	9.0	13.5	0	8.5	54.7
76– 90	3.3	6.7	5.9	6.7	1.7	0	5.1	59.8
91–105	11.0	4.2	0	6.7	1.7	0	5.7	65.5
106–120	13.9	0.8	5.9	6.7	1.7	0	6.2	71.4
121–135	11.5	8.4	2.9	3.4	3.4	11.1	7.5	79.2
136–150	6.2	3.4	5.9	4.5	1.7	0	4.5	83.7
151–165	5.7	4.2	0	1.1	0.	0	3.3	87.0
166–180	6.7	2.9	0	3.4	0	11.1	3.5	90.5
181–195	3.3	0	3.9	0	11.9	0	2.0	93.0
196–210	1.0	0.4	6.9	2.2	3.4	0	1.6	95.0
211–225	0	2.1	2.9	3.4	0	0	.7	96.6
226–240	1.4	1.3	2.0	1.1	3.4	0	.3	98.2
241–255	2.0	0.5	0	0	0 0	0	.7	98.9
256–270	—	—	0	1.1	1.6	0	.3	99.2
271–285	—	—	1.0	1.1	—	0	.3	99.5
286–300	—	—	0	0	—	11.2	.1	99.6
301–315	—	—	1.9	1.2	—	—	.4	100
316–351	—	—	0	0	0	0	—	100

Table 2 shows that the most weevils are found between 31 and 45 feet into the woods. This fact would not have been established by the earlier sampling method with 50-foot intervals. The peak population at this distance into the woods seems to be fairly well established, since both the accumulative average of all six swaths and four of the six swaths taken place the peak populations in this area. Swath F can probably be discounted, since it was sampled after a sustained period of cold weather and the small number of survivors may present an over-emphasized percentage picture. Swath B is particularly noteworthy here, since 27.7% of the 238 weevils taken were found in this 31- to 45-foot span in the brushy top of a fallen tree. However, the tree top may have protected the weevils and raised the survival rate. Then again this brushy material may have intercepted more of the weevils flying into the woods, causing a large number to go into hibernation around it. The latter effect appears possible in the light of findings in these swaths.

Although it is not clear-cut, there is some evidence along other swaths that there is a snow-fence effect with these hibernating weevils; that is, when the weevils fly into the woods to hibernate they fall out on the far side of an intercepting tree or bush and apparently remain there. This interception and subsequent hibernation are further evidenced in swath A. Here the peak hibernating populations were found between 106 and 135 feet—almost 100 feet deeper into the woods than in the other swaths. Trees and bushes are fewest along swath A. Thus there is indication that the weevils will penetrate deeper into the woods if no trees, bushes, or other objects impede their progress.

Also noted in the six swaths was a tendency for peaks to occur every 30 to 35 feet. This might indicate that weevils had a flight range of 30 to 35 feet for each flight. This tendency was noted not only in the individual swaths but in the accumulative average. However, in the consolidated curve the peaks are progressively closer to-

gether as the distance into the woods becomes greater, which may or may not indicate a "tiring" of the flying weevils.

There was no indication that the depth of the trash had any effect on the site of hibernation. Peaks in population occurred in trash from 1/2 to 3 inches deep with no one depth predominating. A definite tendency for more weevils to be found in trash with a high deciduous-leaf and low pine-straw content was established. This trend has been noted in the trash examinations made over the years at the Florence station. Most weevils were found in slightly moist trash, and very few in exceedingly dry or wet material. This observation was also made in other studies of the woods trash-hibernating weevil relationship.

Finally, the data in table 2 indicate that a rather large area would have to be covered with any insecticide or insect pathogen to control hibernating boll weevils. Since such a control would probably kill at best 90% of the weevils reached, to kill 80% of the total weevils present it would be necessary to extend the control into the woods at least 180 feet. Thus every 242 feet of woods edge along a field represents 1 acre of woodland that must be treated. However, with a high population of hibernating weevils the treatment would have to be extended deeper into the woods, since even a low population of weevils in the cotton field in the succeeding crop year has a high reproductive potential. Therefore, owing to the large area that would have to be controlled, the treatment must be inexpensive or effective for several years.

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Biology and Control of *Trogoderma granarium* Everts[1]

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ABSTRACT

The khapra beetle, *Trogoderma granarium* Everts, was first found established in California in 1953 and was subsequently detected in Arizona and New Mexico, as well as in Baja California, Mexico, but has not been found in other states. In connection with the suppression program in progress since 1955, an experimental culture of khapra beetle has been reared in the laboratory under quarantine conditions to develop information as rapidly as possible concerning the biology and control of this insect. Data given here concern food preferences, effect of environmental conditions on the biology of the beetle, and the reaction of starved larvae to low temperatures and fumigation. Results of experiments are given for contact insecticides as grain protectants. Data are presented on fumigation studies as follows: (1) penetration of methyl bromide into the soil; (2) effect of exposures of 2, 8, 24, and 48 hours and temperatures of 50°, 70°, and 90° F. on the control of khapra-beetle larvae by the use of acrylonitrile, ethylene chlorobromide, ethylene dibromide, hydrocyanic acid, and methyl bromide; and (3) fumigation of khapra-beetle larvae at 70° F. with a 24-hour exposure in the presence of no load and of one-half load of wheat of either 7 or 14% moisture content, using acrylonitrile, ethylene chlorobromide, ethylene dibromide, hydrocyanic acid, and methyl bromide.

The khapra beetle was first found established in Tulare County, California in 1953. It is thought to have been present at this location before 1953, however—possibly as early as 1946. Subsequently, it was detected in Arizona and New Mexico, and in Baja California, Mexico. Although inspections for this insect have been conducted in other states, no infestations have been reported. A program of eradication by local, state, and federal authorities was started in 1955 and is continuing. Approximately 300 infestations have been found in California, 200 in Arizona, 6 in New Mexico, and 70 in Mexico. As soon as infestations are detected the premises are fumigated, using 5 pounds of methyl bromide per thousand cubic feet and maintaining a concentration above 32 ounces per thousand cubic feet at all sampling points for not less than an aggregate of any 24 hours, or 24 ounces for 36 hours, out of a continuous 48-hour or longer period. This suppression program to date appears to be highly successful but is dependent upon the thoroughness and speed with which infestations are located and treated.

With the approval of local, state, and federal agencies, an experimental culture of khapra beetle has been reared under quarantine conditions at the University of California Citrus Experiment Station, Riverside, since 1953. The work reported here is a continuation of that reported by Lindgren *et al.* in 1955.

BIOLOGY.—*Food Studies.*—Experiments were conducted with various available food products to determine whether they were favorable to the development of khapra beetle. Two series of experiments were conducted at 90° F. In the first series, adult beetles were introduced into each type of food; in the second series, larvae were introduced. Observations were made weekly to check development. Some feeding on dead insects was observed in this study which may have influenced the results. Where a complete generation was obtained, the time interval for a single generation ranged from 1 to 2½ months, the length of the interval depending on the type of food present. Complete generations were obtained on 23 of the 39 foods tested (table 1). In some cases, on those foods in

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[2] The authors are indebted to the personnel of county, state, and federal agencies for their many suggestions pertaining to this investigation.

Table 1.—**Effect of food on development of the khapra beetle at 90° F.**

GENERATION COMPLETED IN LESS THAN 2.5 MONTHS	GENERATION NOT COMPLETED IN 6 TO 12 MONTHS
Barley, rolled	Chocolate chips
Blackeye cowpeas	Chocolate, powdered
Bran	Dates, whole
Coconuts	Dates, ground
Corn, whole	Figs, dried
Corn meal	Nutmeg
Cotton seed, fuzzy	Peaches, dried
Egg noodles	Peaches, ground dried
Flax, ground	Pears, dried
Flour, bleached white	Prunes, dried
Flour, whole wheat	Prunes, ground dried
Garbanzos	Raisins, whole
Hominy grits	Raisins, ground
Lentils	Sugar, brown
Milk, powdered skim	Sugar, white
Oats, whole	Tapioca
Oats, rolled	
Orange pulp, dried	
Peanuts, shelled	
Peas, dried	
Spaghetti	
Yeast, powdered	
Wheat germ	

which no generation was completed, no larvae, or very few larvae, were present; in other cases numerous larvae were present but pupation did not occur during the period of observation.

Various foods (table 2) were finely ground with a mortar and pestle and weighed on an analytical balance to 2, 4, 6, and 8 mg. Each food sample was placed in a plastic cell with a single khapra-beetle egg and replicated four times. These cells were held at a temperature of 90° F. and 50% relative humidity. Observations on development were made at weekly intervals, but the sex of the emerging adults was not determined. Shed skins were removed at each observation. Under these conditions the least amount of food required to produce a single adult khapra beetle was 4 mg; with certain other foods 8 mg. was not enough.

A study was made to determine the gross food consumption by successive series of generations of khapra beetle. The foods used were sifted through a 20-mesh screen allowing the finer materials to pass through. A 50-gm. aliquot was taken from the coarse material that remained in the screen. Fifty third- or fourth-instar khapra-beetle larvae were placed in a ½-pint Mason jar containing the 50-gram food aliquot, and were held at various temperatures. At intervals the entire food aliquot was sifted. The frass passed through the screen and was weighed, and the coarse material that did not pass through the screen along with the insects was held for subsequent observations. At the termination of the experiment the food remaining was weighed.

By taking the total food consumed, which is equal to the total food initially used minus the remains at the end of the experiment, and dividing this by the total weight of frass, the approximate amount of food consumed per unit weight of frass was obtained. This figure multiplied by 2 gave the percentage of food consumed.

Several generations of insects developed during the progress of the experiment and although at each sifting the khapra beetles were carefully replaced in the remainder of the food, a few of the earlier stages were undoubtedly lost.

Table 2.—**Minimum amount of various foods required for development of the khapra beetle.**

FOOD	AMOUNT (MG.)	STAGE OF DEVELOPMENT
Barley flour	8	adult
Corn meal	8	larva
Dog food	4	adult
Milk, dried	6	adult
Pinto bean flour	6	adult
Rice flour	8	larva
Rolled oat flour	6	adult
Whole wheat flour	4	adult

As shown in table 3, the higher the temperature was, the more rapidly the food was consumed. With some foods, 90% was consumed in four months at 100° F.; with others, less than 40% was consumed under the same conditions. This indicates a definite food preference.

The number of khapra beetles present when these materials were sifted varied greatly owing to the type of food available to them. In whole or cracked wheat heavy populations developed rapidly, whereas in other foods such as beans and flax few khapra beetles were present.

Resistance of Eggs to High Temperature.—Tests were conducted to determine the thermal death point of eggs of khapra beetle at several humidities. Methods used were similar to those described by Lindgren *et al.* (1955). From the results shown in table 4 it may be noted that relative humidity had very little effect on the susceptibility of khapra-beetle eggs to high temperatures. An increase of 6 degrees, from 120° to 126° F., greatly reduced the time required to kill 50% or 95% of the eggs, regardless of relative humidity. At a temperature of 135° F., 95% of the khapra-beetle eggs were killed in 3 minutes.

Effect of Fluctuating Temperatures on the Life History of the Khapra Beetle.—A study to determine the effect of fluctuating temperatures on the life history of the khapra beetle was undertaken. Although this experiment has not been terminated, the incomplete results are of sufficient value to be presented. The results definitely indicated the presence of both short- and long-lived individuals.

All experiments were conducted at 50% relative humidity. Temperatures were maintained by using small temperature-controlled chambers placed in rooms having air temperatures of either 55° or 70° F. A time clock operated a thermostatically controlled heat unit in each chamber for the high temperature period. When the time clock turned off the current for the thermostatically controlled heat source, the temperature in the cabinet returned to room temperature.

Individual insects were reared in cells containing a surplus of cracked wheat as food. Table 5 gives results with eight individuals per test. Natural mortality was very low. In table 5 are also included, for comparative purposes, the results of previous studies at constant temperatures of 70°, 80°, and 90° F. In comparing the average temperatures of the fluctuating-temperature experiments it will be noticed that the time required for development from egg to adult insect was generally less

Table 3.—Effect of temperature on food consumption by the khapra beetle.[a]

Food	Temp., ° F.	Per Cent of Total Food Consumed in Months Shown						Per Cent Food Not Consumed
		2	4	5	8	10	13	
Whole wheat	80	14.4	38.9	77.8	90.4	—	—	9.6
	90	18.4	66.2	86.8	90.4	—	—	9.6
	100	22.6	89.2	92.0	93.6	—	—	6.4
Cracked wheat	80	25.6	82.4	89.6	92.6	—	—	7.4
	90	42.2	87.4	89.8	92.8	—	—	7.2
	100	47.6	90.0	92.4	95.2	—	—	4.8
Whole barley	80	10.1	21.9	25.7	29.5	—	—	70.5
	90	13.2	27.0	31.6	35.6	—	—	64.4
	100	22.2	35.0	39.0	42.4	—	—	57.6
Rolled barley	80	24.1	73.0	79.0	82.8	—	—	17.2
	90	35.1	73.0	77.2	80.0	—	—	20.0
	100	45.5	78.4	82.6	86.1	—	—	13.9
Whole corn	80	7.5	22.5	32.5	50.0	—	—	50.0
	90	12.5	31.5	45.0	59.0	—	—	41.0
	100	19.0	40.2	59.8	68.2	—	—	31.8
Cracked corn	80	28.6	37.2	47.2	54.6	—	—	45.4
	90	26.8	39.2	49.4	56.8	—	—	43.2
	100	33.4	51.8	66.6	71.4	—	—	28.6
Flax	100	—	15.2	—	—	28.4	30.0	70.0
Rolled oats	100	—	67.0	—	—	98.2	98.6	1.4
Whole oats	100	—	8.2	—	—	8.2	11.6	88.4
Rice, polished	100	—	75.4	—	—	93.2	96.4	3.6
Rice, unpolished	100	—	89.0	—	—	97.8	99.4	0.6
Sorghum	100	—	30.8	—	—	89.8	95.2	4.8
Beans, black-eyed	100	—	18.0	—	—	18.8	19.6	80.4
Beans, pinto	100	—	6.2	—	—	11.0	11.6	88.4
Beans, lima	100	—	16.0	—	—	24.4	27.6	72.4
Beans, soya	100	—	5.2	—	—	9.6	11.0	89.0
Peas, whole	100	—	4.8	—	—	6.4	8.0	92.0
Lentils	100	—	13.2	—	—	16.4	17.2	82.8
Garbanzos	100	—	17.2	—	—	21.8	29.2	70.8

[a] Initial infestation, 50 larvae.

than that for the corresponding constant temperature. A temperature of 100° F. on the high cycle appeared to be slightly above the optimum, but considerably more work should be done on the effects of fluctuating temperatures on the life history of the khapra beetle particularly with reference to the average time required from egg to adult for both short- and long-lived individuals.

Limited studies conducted to determine the number of generations per year of khapra beetle in a protected, shady area at Riverside, California, indicated that when this insect was placed on ground cracked wheat, approximately two generations were completed in a year. In this study one generation was completed during the period from the first of June to the middle of August, and a second generation was completed between the middle of August and the middle of the following July.

Effect of Carbon Dioxide on Khapra-Beetle Larvae.—Results of exposure of third- and fourth-instar khapra-beetle larvae for various periods of time to a relatively pure atmosphere of carbon dioxide were conducted to determine mortality. Larvae were placed in pint Mason jars. The atmosphere within each jar was displaced for 3 minutes with carbon dioxide. The jars were then sealed and kept at room temperature for various lengths of time. A control was held under identical conditions but without the addition of carbon dioxide. There was some survival of larvae under these conditions up to 14 days' exposure, but no survival at 19 or more days' exposure.

Resistance of Starved and of Fed Khapra-Beetle Larvae to Low Temperatures and Fumigation.—Large numbers of third- and fourth-instar larvae were placed in containers without food and held at a temperature of 90° F. The pos-

Table 4.—**Effect of relative humidity on time required to kill 50% and 95% of khapra-beetle eggs at high temperatures.**

Temperature (° F.)	Relative Humidity (%)	Minutes Required to Kill Eggs	
		50%	95%
120	25	100	228
	50	100	228
	75	130	232
126	25	<5	20
	50	<5	20
	75	<5	20
131	25	<4	5
	50	<4	5
	75	<4	5
135	25	<2	3
	50	<2	3
	75	<2	3

sibility of cannibalism existed, but no observations were made to determine this. The starved larvae were generally much smaller than the fed larvae. Fed larvae and larvae starved for 27 and 254 days were held at temperatures ranging from 14° to 20° F. Aliquots were removed at various intervals and held for 20 days for mortality checks. Results are given in table 6. In general, the starved larvae were more resistant to low temperatures than the fed larvae. Larvae starved for 254 days appeared to be more resistant to low temperatures than those starved for 27 days.

Similar tests were undertaken to determine the effect of starvation of khapra-beetle larvae on fumigation results. Fumigations were conducted for 2 hours at 75° F. in a gastight chamber in which the air was circulated for a short time following application of the fumigant. Approximately 50 khapra-beetle larvae of each starvation period were used in each test, and all starvation groups were represented in each fumigation. At least three or more points were obtained to establish a curve from which the LD_{50} could be determined. Results are given in table 7. Under these conditions it appeared that starvation had little or no effect on larvae fumigated with acrylonitrile; however, starved larvae appeared to be less susceptible than fed larvae to fumigation with hydrocyanic acid, ethylene dibromide, and methyl bromide.

Effect of Environmental Conditions on Khapra-Beetle Larvae.—A ½-inch transite cylinder 14 inches in diameter and 4 feet high was sealed at the bottom and filled with wheat of 12% moisture content to a depth of 46 inches. Thermocouples were placed in the center of this column of wheat at 4-inch intervals. This cylinder was placed in a room in which the air temperature was controlled to

Table 5.—**Effect of fluctuating temperatures on the life history of the khapra beetle.**

Exposure Period, per Day (Hours)	Temperature (° F.)	Average Temperature (° F.)	Total Days Egg to Adult, Range[a]
11	55	68.5	124–295[b]
13	80		
11	55	74.0	49–171
13	90		
11	70	75.4	79–281[b]
13	80		
16	70	76.7	52– 83[b]
8	90		
11	55	79.4	59–329[b]
13	100		
11	70	80.8	35– 45
13	90		
8	70	83.3	25– 46
16	90		
11	70	86.3	29– 35
13	100		
8	70	90.0	32– 81
16	100		
24	70	70	158–310
24	80	80	63–216
24	90	90	28– 56

[a] The range in days of development is given as an infestation of khapra beetles includes two types of larvae—a long-lived and a short-lived. In these studies, in which a limited number of individuals were used, it was obvious from the results obtained that the two groups of larvae were present.

[b] Larvae present.

Table 6.—**Effects of starvation and of exposure for various periods of time to fluctuating temperatures (14° to 20° F.) on percentage mortality of khapra-beetle larvae.**

Exposure to Fluctuating Temperatures (Days)	Starvation Period (Days)	Per Cent Larval Mortality
8	0	0
	27	0
	254	20
15	0	10
	27	0
	254	10
22	0	80
	27	20
	254	10
29	0	90
	27	60
	254	30
36	0	100
	27	80
	254	40
43	0	90
	27	70
	254	60
50	0	100
	27	100
	254	50
57	0	100
	27	90
	254	40

Table 7.—Effects of starvation of khapra-beetle larvae for various lengths of time on fumigation results.[a] (2-hour exposure at 75° F.)

Larvae Starved (Days)	Milligrams of Fumigant per Liter to Give 50% Kill (LD_{50}) of Larvae			
	Acrylonitrile	Ethylene Dibromide	Hydrocyanic Acid	Methyl Bromide
0	8.25	19.9	0.8	31.0
22– 24	—	28.0	5.4	38.0
50– 58	10.0	29.3	5.2	41.0
77– 79	9.5	28.0	5.4	45.0
93– 99	—	30.7	5.0	45.0
106–134	10.5	29.5	6.0	46.0

[a] Two-hour exposure at 75° F.

Table 8.—Number of khapra-beetle larvae present and evidence of food consumption in probe samples from various depths in a column of wheat under controlled conditions.

Depth of Sample (Inches)	No. of Larvae in Sample	Wheat (Gm.)	Screenings (Gm.)
2	146	2	0.2
6	190	18	5.9
10	126	38	3.8
18	55	45	1.4
22	20	47	0.5
26	10	46	0.3
34	7	47	0.2
38	7	47	0.2
42	13	44	0.2

±1° F. On January 25, 1956, approximately 100,000 khapra-beetle larvae were introduced onto the surface of the wheat. The cylinder was then sealed at the top with a fine-mesh screen to prevent escape of the insects. Temperature readings were taken at weekly intervals. The room in which the cylinder was placed was held at the following temperatures: 80° F., January 24 to October 3, 1956; 65° F., October 3 to October 12, 1956; 80° F., October 12 to November 29, 1956; and 100° F., November 29, 1956, to April 3, 1957.

The temperatures at the various depths within the column are shown in figure 1. On February 2, 1956, larvae were found at the 38-inch depth. During the intervals when the room temperature in which the cylinder was held was 80° F., the temperature at the 2- and 6-inch depths reached 102° F. When the room temperature was dropped to 65° F., the temperature within the column of wheat at the various depths dropped to 70° F. or lower and remained at this temperature until the room temperature was again changed to 80° F. This indicates that the insects under these conditions were not able to maintain a temperature which would allow them to remain active, probably because the small mass of wheat present did not afford enough insulation. Temperatures of 105° to 107° F., were recorded at the 6-inch depth when the room temperature was 100° F.

At the end of the experiment, a probe was used to obtain 52-ml. samples of infested wheat at various depths. Each sample was examined as to number of larvae present, weight of wheat, and weight of fine screenings (mostly frass). Results are given in table 8. At the 6-inch depth the sample contained 190 larvae, while at the 38-inch depth only seven larvae were present. At the 2-inch depth there were 2 grams of partially chewed wheat in the sample, while at the 38-inch depth there were 47 grams of unchewed wheat. At the 6-inch depth there were 6 grams of screenings, while at the 38-inch depth there was 0.2 gram.

Effect of Relative Humidity on the Biology of the Khapra Beetle.—Khapra beetles were reared at 95° F. in desiccators in which the relative humidity was controlled with either potassium hydroxide solution or sulfuric acid solu-

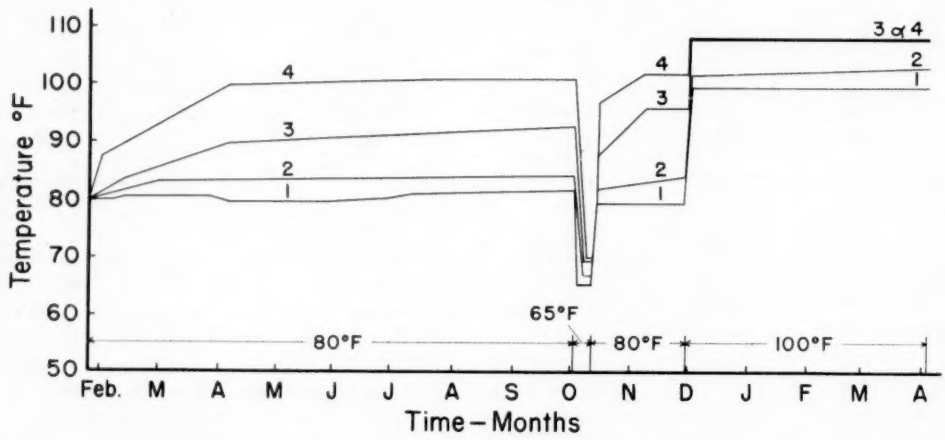

FIG. 1.—Effects of the khapra beetle on temperatures at various depths within a column of grain.

Room temperatures: 80° F., January 24 to October 3, 1956; 65° F., October 3 to October 12, 1956; 80° F., October 12 to November 29, 1956; 100° F., November 29, 1956, to April 3, 1957. Temperatures at various depths within a column of grain (khapra-beetle larvae introduced onto surface of grain January 25, 1956):

Curve 1) at depths of 22, 26, 30, 34, 38, 42, and 46 inches.

Curve 2) at depths of 14 and 18 inches.

Curve 3) at a depth of 10 inches.

Curve 4) at depths of 2 and 6 inches.

tion, under conditions of 50% darkness and 50% light. Calcium sulfate was used to obtain approximately 0% relative humidity. A food supply consisting of whole wheat flour was placed in containers in the desiccator prior to the introduction of adult beetles, to allow the food to come into equilibrium with the relative humidity in the desiccator. Adults were introduced and allowed to lay eggs for 1 week; the adults were then removed. Observations were made approximately every 3 to 4 days until the first adult appeared and was recorded; the experiment was then terminated. The data on egg viability and length of life cycle are given in table 9. There was very little difference in egg viability at relative humidities of 10, 35, or 65%, but at the extremes (0 and 90% relative humidity) there was a slight reduction in viability.

The average minimum number of days required for development from egg to adult varied only slightly at 10, 35, 65, and 95% relative humidities, but at 0% relative humidity it appeared to take slightly longer than at the other relative humidities. In a few tests several consecutive generations were allowed to develop at the various relative humidities. Results indicated that under these conditions the insects could survive and reproduce. Egg production at 0% relative humidity and 95° F. ranged from 31 to 88 eggs per female.

Attempts were made to rear khapra beetles on growing wheat in the greenhouse without success. Larvae were placed on the heads of growing and fully ripened grain but eventually dropped off; other larvae were placed at the base of stems, but did not attempt to crawl up the stalk. Although these tests indicated that the khapra beetle did not feed and establish itself on growing wheat, more extensive tests should be conducted to substantiate these results.

CONTROL.—*Contact Insecticides as Grain Protectants for Control of Khapra Beetle.*—Wheat of 10 and 14% moisture content was sprayed with either 8 or 16 parts per million of allethrin, malathion, chlorthion, aldrin, lindane, DDD, DDT, and Pyrocide 175 (20% pyrethrins). One hundred mature khapra-beetle larvae were placed in an aliquot of treated wheat 1 day after treatment and left for a period of 30 days. With the exception of malathion, none of the materials were effective under the conditions of these tests.

Fumigation Studies.—A hole 6 inches in diameter and 3 feet deep was dug in the center of a 10-foot-square level plot of sandy loam. Third- or fourth-instar khapra-beetle larvae in metal probes were placed in the hole at depths of 30, 21, 15, 9, and 3 inches. Each probe was covered with soil as it was placed in the hole, and the soil was tamped. Air temperatures ranged from 54° F. at night to over 100° F. during the day. The soil was dry, and soil temperatures were not obtained. The plot was covered with a gastight polyethylene tarpaulin which was sealed in a 3-inch-deep trench with sand. An air headspace of approximately 6 to 10 inches was maintained between the tarpaulin and the top of the soil. Methyl bromide was applied in a flat container to facilitate rapid evaporation. The exposure period was 24 hours.

Data given in table 10 show that, at a dosage of 0.4 pound methyl bromide per 100 square feet, kill of the larvae was incomplete at the 30-inch depth, but at a 1-pound dosage the percentage of kill was 100.

Table 9.—Effect of relative humidity on the number of days required for development of the khapra beetle from egg to adult, and on the viability of the eggs.

Per Cent Relative Humidity	Average Minimum Days, Egg to Adult	Per Cent Egg Hatch
0[a]	42	92
10	32	100
35	27	100
65	31	95
90	31	85

[a] Approximate relative humidity obtained with calcium sulfate.

The lowest dosage to kill 100% of mature khapra-beetle larvae under conditions of no load was determined for acrylonitrile, ethylene chlorobromide, ethylene dibromide, hydrocyanic acid, and methyl bromide at exposures of 2, 8, 24, and 48 hours and temperatures of 50°, 70°, and 90° F. Fifty or more larvae were used per test, and individual tests were conducted until the 100% point was determined and could be replicated three times; however, with a larger number of individuals and a greater number of replications there is no doubt that an occasional khapra-beetle larva would survive.

Temperature was controlled to within ±1° F., and air was circulated in the gastight 1-cubic-foot chamber for a short time following application of the fumigant. The fumigated larvae were held for 10 days at 80° F., at which time mortality counts were made. There was no natural mortality. The results of these experiments are given in table 11. It will be noted that the lower the temperature was, the greater was the dosage required to obtain 100% kill, and it would appear that the effect of temperature on the kill was greater between temperatures of 50° and 70° F., than between those of 70° and 90° F. It will also be noted that the longer the exposure period was, the greater was the kill.

Table 12 gives the ratios of time and dosage necessary to obtain 100% (complete) kill of khapra-beetle larvae at various temperatures and exposures. The ratios were obtained by graphing on logarithmic paper the pounds per 1,000 cubic feet versus time to give 100% kill at temperatures of 50°, 70°, and 90° F. The horizontal or vertical differences between the lines on the graphs give the ratio required at any time or dosage for 100% (complete) kill. For example, when acrylonitrile is used, and a 2-hour exposure, the ratios are 1 at 90° F., 1.5 at 70°, and 3 at 50°,

Table 10.—Mortality of khapra-beetle larvae at various depths in soil fumigated with methyl bromide.

Methyl Bromide Dosage (Pounds per 100 Sq. Ft.)	Per Cent Kill of Khapra-Beetle Larvae in Fumigated Soil at Depths Shown (In.)				
	3	9	15	21	30
0.05	100	100	77.2	40.7	0
.1	100	100	100	83.1	38.5
.15	100	100	100	100	74.2
.2	100	100	100	100	79.6
.3	100	100	100	94.5	41.8
.4	100	100	100	100	72.7
1.0	100	100	100	100	100

Table 11.—Results of atmospheric fumigation of khapra-beetle larvae with various fumigants and at various temperatures and exposure periods.

Fumigant	Exposure Period (Hours)	Lowest Dosage (Lb./1,000 Cu. Ft.) to Give 100% Kill of Larvae		
		50° F.	70° F.	90° F.
Acrylonitrile	2	2.7	1.2	1.0
	8	0.89	0.71	0.51
	24	0.53	0.22	0.21
	48	0.27	0.14	0.17
Ethylene chlorobromide	2	>18.9	11.3	4.0
	8	9.4	5.3	3.0
	24	6.0	2.3	1.5
	48	3.0	1.7	1.3
Ethylene dibromide	2	6.8	3.87	2.3
	8	3.9	0.97	0.92
	24	1.7	0.5	0.23
	48	0.78	0.29	0.19
Hydrocyanic acid	2	1.1	0.77	0.25
	8	0.55	0.2	0.13
	24	0.16	0.1	0.06
	48	0.07	0.03	0.04
Methyl bromide	2	11.6	3.87	2.6
	8	5.4	1.6	0.77
	24	1.4	0.53	0.52
	48	1.2	0.47	0.45

indicating that if at a given dosage it required 2 hours at 90° F. (1×2), it would require 3 hours at 70° (1.5×2) and 6 hours at 50° (3×2) to give 100% (complete) kill; or, on a dosage basis, using acrylonitrile as an example and a 2-hour exposure, if a unit of 1 in dosage measurement gives 100% kill at 90° F., it would require 1.3 times this amount at 70°, and 2.3 times this amount at 50° to obtain 100% kill.

A series of experiments was conducted to determine the relative toxicity of various fumigants to mature khapra-beetle larvae in the presence of wheat, at the surface and at two different depths below the surface. Tests were made in gastight fumigation chambers of 28-liter volume. These chambers were loaded with clean wheat of 7 or 14% moisture content. Fifty or more khapra-beetle larvae in probes were placed in the wheat at the following positions: 1 inch from the bottom, 4 inches from the bottom, and at the surface, which was 7 inches from the bottom, thus allowing a 7-inch headspace. The fumigants were applied with a microburette to small pieces of filter paper. The chambers were then sealed and held for an exposure of 24 hours at 70° F. The fumigated insects were held at 80° F. for 10 days, at which time mortality counts were made. Dosage applied in pounds per 1,000 cubic feet was computed on the total volume of the fumigation chamber, which was 1 cubic foot.

Table 13.—Lowest dosages of fumigants to give 100% (complete) kill of khapra-beetle larvae in wheat.[a]

Fumigant	Dosage (Lb./1,000 Cu. Ft.) for 100% Kill of Larvae			
	One-Half Load Wheat			
	Position of Larvae	Moisture Content		No Load Wheat
		7%	14%	
Acrylonitrile	Surface	0.53	0.71	0.22
	Center	0.89	4.5	
	Bottom	0.89	8.0	
Ethylene chlorobromide	Surface	9.4	11.3	2.3
	Center	13.2	18.8	
	Bottom	>23.0	>30.0	
Ethylene dibromide	Surface	3.6	3.9	0.5
	Center	8.4	14.5	
	Bottom	12.1	>29.0	
Hydrocyanic acid	Surface	0.23	0.46	0.1
	Center	0.46	1.54	
	Bottom	0.61	4.3	
Methyl bromide	Surface	0.86	0.92	0.53
	Center	0.86	1.1	
	Bottom	1.0	1.24	

[a] Exposure, 24 hours; temperature, 70° F.

The lowest dosages in pounds per 1,000 cubic feet to give 100% kill of khapra-beetle larvae for no load and for one-half load of wheat are shown in table 13. It may be noted that in all cases a higher dosage was required to kill 100% of the khapra-beetle larvae in one-half load of wheat than with no load, and that the higher the moisture content of the wheat was, the greater the dosage required; however, this difference was very small in the

Table 12.—Ratios of time and dosage necessary for 100% (complete) kill of khapra-beetle larvae with various fumigants at various temperatures (° F.) and exposures.[a]

Exposure (Hours)	Ratio for	Acrylonitrile			Ethylene Chlorobromide			Ethylene Dibromide			Hydrocyanic Acid			Methyl Bromide		
		90°	70°	50°	90°	70°	50°	90°	70°	50°	90°	70°	50°	90°	70°	50°
2	Time	1	1.5	3.0	1	2.7	6.8	1	1.6	5.5	1	1.9	4.7	1	1.9	8.5
	Dosage	1	1.3	2.3	1	2.0	4.0	1	1.4	3.2	1	1.8	3.7	1	1.5	4.5
12	Time	1	1.25	2.8	1	2.3	5.5	1	1.6	7.3	1	1.7	4.0	1	1.7	7.5
	Dosage	1	1.1	2.2	1	1.8	3.4	1	1.5	4.4	1	1.5	3.3	1	1.4	4.2
48	Time	1	1.2	2.6	1	2.1	5.4	1	1.5	9.0	1	1.4	3.2	1	1.6	6.6
	Dosage	1	1.0	2.0	1	1.6	3.1	1	1.5	5.6	1	1.2	3.1	1	1.3	3.8

[a] Data from table 11.

The fumigated insects were held at 80° F. for 10 days, at which time mortality counts were made. Dosage applied in pounds per 1,000 cubic feet was computed on the total volume of the fumigation chamber, which was 1 cubic foot.

case of methyl bromide. The amount of fumigant required to kill 100% of the khapra-beetle larvae at the lowest depth was greater than that required to give a similar kill at the surface; but again, this difference in the case of methyl bromide was very slight.

REFERENCE CITED

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Effect of Methyl Bromide and Hydrocyanic Acid Fumigation on the Germination of Barley[1]

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ABSTRACT

Four varieties of barley (Atlas 46, California Mariout, Hannchen, and White Smyrna) with a graded moisture range of 8, 10, 12, and 14% were fumigated with methyl bromide in 100-cubic-foot gastight chambers at an exposure period of 72 hours. Various dosages of fumigant and temperatures of 50, 70, and 90° F. were used. One variety, California Mariout, was fumigated with methyl bromide and hydrocyanic acid under varying conditions of temperature, exposure, and dosages of fumigant. One-half of the fumigated samples of seed received one fumigation; the others were fumigated twice. Two series of germination tests were made, the first within 5 days following fumigation of seeds, and the second after 84 days of postfumigation storage at 50° F.

Under the conditions of these studies, the germination of barley was not affected by fumigation with hydrocyanic acid. Injury resulting from methyl bromide fumigations could be identified as dead seed. Little evidence of retarded emergence was observed. Dosage, temperature, period of exposure, and moisture content of seeds were variables found to be most important in contributing to injury from methyl bromide. However, barley was highly tolerant to methyl bromide fumigations under the most extreme conditions.

Fumigation for the eradication of a storage pest with a host range so broad as that of the khapra beetle, *Trogoderma granarium* Everts, presents the problem of possible injury to plant seeds of innumerable species. Tolerance of cereal grain seeds to fumigants was of immediate concern in the California Khapra Beetle Eradication Program.

It is the purpose of this paper to evaluate several factors which may influence the germination of barley fumigated with methyl bromide or hydrocyanic acid. Results reported were obtained from studies made under controlled conditions with proven techniques. Germination of barley was not impaired after fumigation with hydrocyanic acid and a high degree of tolerance to methyl bromide was demonstrated.

MATERIALS AND METHODS.—*Preparation of Seed Samples.*—Four varieties of barley (Atlas 46, California Mariout, Hannchen, and White Smyrna) were used in one series of tests (72-hour exposure period) with methyl bromide. California Mariout was selected as a representative variety for tests throughout the study. The initial moisture content (m.c.) of barley seeds was as follows: Atlas 46—8.6%, California Mariout—8.0%, Hannchen—8.5%, White Smyrna—9.0%. The moisture content of California Mariout seeds ranged from 8.0 to 10.5% due to normal fluctuations in storage during the experimental period of approximately 18 months.

Careful adjustment of moisture and conditioning of seeds resulted in the desired graded moisture range of 8, 10, 12, and 14%. Usually it was necessary to dry seeds, add water, or alternate drying and addition of moisture to obtain samples of the desired moisture content.

Seeds were dried in narrow wire-screen containers placed in a forced-air oven maintained at 125 to 130° F. This temperature did not affect the germination of barley. The drying period ranged up to 3 hours at any one time; never longer. The moisture content of seeds before drying and the desired moisture level indicated the length of time seeds were to be held in the air oven. Water was added to increase the moisture content. After drying or addition of water, seeds were conditioned in airtight polyethylene bags for 7 days at 50° F. Then seeds were blended and moisture tests were made to ascertain the need for additional moisture adjustments. The moisture content of seeds was determined by the air oven method specified in the Association of Official Agricultural Chemists (A.O.A.C.) Official Methods of Analysis (Horwitz 1955).

When the moisture content of barley had reached the desired level, each lot of seeds was divided into samples of approximately 100 grams each and put into small rough-woven cotton bags. Seeds were removed from these bags only for germination tests. Each cloth sample-bag of barley was placed in an airtight polyethylene bag to prevent moisture changes while the prepared samples awaiting fumigation were held in storage at 50° F.

Fumigation of Seeds.—Test samples were removed from storage and held overnight at the desired fumigation temperature before removing airtight polyethylene bags. Seeds of all four moisture levels were placed in slatted crates inside the fumigation chambers which contained only the samples to be fumigated together. The crates permitted thorough circulation of gases.

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Fumigation experiments were conducted in 100-cubic-foot gastight chambers with continuous circulation of air within the fumatorium. Temperatures were thermostatically controlled at 50, 70, and 90° F. ± 1° F., but no attempt was made to control humidity. Fumigations were twice replicated.

Fumigants were applied at two dosage levels, one high and one low, throughout the study. Dosages were selected according to exposure periods and temperatures during fumigation. Seeds were exposed to fumigants for 72 hours in beginning tests. When germination was impaired at this exposure from the dosages applied, tests were made with shorter exposure periods.

The exposure periods, temperatures during fumigation, and dosages of fumigants applied are given in table 1. Concentrations of fumigants were not determined for each dosage or exposure interval. However, typical curves for the fall in concentrations of methyl bromide were given previously by Strong & Lindgren (1959).

All seeds received an initial fumigation. One-half of the samples were fumigated a second time 21 days after the initial exposure to test the effect of repeat fumigation. After fumigation, seeds were aerated for 24 hours before repackaging in airtight polyethylene bags. Test samples were held in postfumigation storage at 50° F. and removed only for germination tests.

Germination Tests and Assessment of Results.—One series of germination tests was made within 5 days after fumigation of seeds and another after 84 days (12 weeks) in postfumigation storage at 50° F. Seeds were germinated at 68° F. in folded paper towel substrata.

Germination tests and seedling interpretations were made in accordance with regular methods of Official Seed Analysts (Assoc. Off. Seed Analysts 1954) except seeds with obvious mechanical injury were not planted, and 100 seeds were used per test for each treatment replicate. Nonfumigated seeds served as control standards for comparison.

The percentage of live seeds (normal and abnormal seedlings) and the percentage germination (normal seedlings) were the essential criteria recorded for comparison of results. (See p. 131, U. S. Dept. Agric. Agricultural Handbook No. 30, 1952, for a description of normal and abnormal seedlings.) The distinction made between germinated seeds and live seeds was considered of considerable importance since fumigants may cause injury by retarding germination as well as destruction of germinative capacity.

RESULTS.—Due to the enormous number of tests (2,160 germination tests of 100 seeds per test) made during the study, it has been necessary to materially condense results and present only a summary of data essential to the understanding and discussion of factors investigated. The differences between percentages of germinated and live seeds were negligible, indicating little retarding of germination due to fumigation. Thus, results from fumigated seeds are presented only as the corrected percentages of reduction in germination. These were calculated by extending Abbot's (1925) formula as follows: Corrected Percentage Reduction in Germination =

$$\frac{\%\text{ germination in control samples} - \%\text{ germination in fumigated samples}}{\%\text{ germination in control samples}} \times 100$$

Table 1.—Exposure periods, temperatures, and dosages of hydrocyanic acid and methyl bromide used for the fumigation of barley seeds.

Fumigant	Exposure (Hours)	Temperature (° F.)	Dosage (Lb./1000 Cu. Ft.) Low	High
Hydrocyanic acid	72	90	1.0	2.0
		70	1.0	2.5
		50	1.0	3.0
Methyl bromide	72	90	1.0	3.0
		70	1.5	3.0
		50	2.0	4.0
	48	90	1.5	4.0
		70	2.0	4.0
		50	2.5	5.0
	24	90	1.5	4.0
		70	2.0	4.0
		50	2.5	4.0
	8	90	2.0	5.0
		70	3.0	5.0
		50	3.0	6.0
	2	90	3.0	6.0
		70	3.0	6.0
		50	3.0	6.0

The actual percentages of germination in control samples of four varieties of barley used for the 72-hour exposure period to methyl bromide are summarized in table 2. The frequency at which percentages of germination occurred refers to the actual number of tests with a given percentage germination out of 96 tests for each variety. It is readily seen that seeds of all varieties were of comparable high quality. Similarly, results from germination tests with control samples of California Mariout barley are summarized in table 3. The frequency at which percentages of germination occurred are given for each graded moisture range to show that procedures followed in the preparation of seed samples did not affect germination.

Hydrocyanic Acid Fumigation.—Exposure of California Mariout barley with a graded moisture range of 8, 10, 12, and 14% to hydrocyanic acid fumigation for 72 hours at temperatures and dosages listed in table 1 did

Table 2.—Frequency[a] at which percentages of germination occurred in unfumigated control samples of four varieties of barley for the 72-hour exposure period to methyl bromide fumigation.

Variety of Barley	Per Cent Germination 95	96	97	98	99	100
Atlas 46	1	10	7	35	31	12
California Mariout	—	4	8	27	38	19
Hannchen	1	3	14	27	31	20
White Smyrna	—	—	9	15	42	30

[a] Expressed as the number of times in 96 germination tests for each variety.

Table 3.—Frequency[a] at which percentages of germination occurred in unfumigated control samples of California Mariout barley for all fumigation tests.

Per Cent Moisture Content	Per Cent Germination							
	93	94	95	96	97	98	99	100
8	1	1	0	2	13	32	44	51
10	0	0	0	6	5	44	48	41
12	0	0	2	7	12	35	53	35
14	0	0	0	4	11	40	50	39

[a] Expressed as the number of times in 144 germination tests for each moisture range.

not result in significant reduction in germination. Thus, only a summary of results for the extremes in treatments is given in table 4. It is obvious from the data presented that further discussion is not justified.

Methyl Bromide Fumigation.—A summary of results from the first series of germination tests (seeds planted within 5 days after fumigation) are presented in tables 5 to 8 as the corrected percentages of reduction in germination (given as nearest whole number). Although barley was found to be quite tolerant to methyl bromide as compared with wheat (Strong & Lindgren 1959), differences between treatments indicate that several variables considered in this study contributed to some reduction in germination after fumigation with methyl bromide. This is discussed more fully below.

Discussion of Results from Methyl Bromide Fumigations.—Seven factors were considered of primary importance in this study. These are listed and discussed separately in regard to the importance of each variable in contributing to the injury of barley seed from methyl bromide fumigation.

Varietal Differences.—The four varieties of barley used in one series of tests (72-hour exposure period) with methyl bromide were grown in different areas of California, so, in addition to varietal differences, the cultural background of the seeds might have an influence on the final results. However, as illustrated in table 2, all four varieties of barley were of comparable high quality. All varieties demonstrated considerable tolerance to methyl bromide when fumigated under extreme conditions (table 5).

It is difficult to attribute much importance to varietal susceptibility to methyl bromide from the small differences observed between varieties in table 5. Rather, the results tend to agree with those reported by Lubatti & Blackith (1957) from germination tests following fumigation with methyl bromide and suggests that the main dif-

Table 4.—Corrected percentages of reduction in the germination of California Mariout barley after two fumigations with hydrocyanic acid for 72 hours at three temperatures.

Per Cent Moisture Content of Seeds	Fumigation Temperature and Dosage in Lb./1000 Cu. Ft.		
	90° F.—2.0	70° F—2.5	50° F.—3.0
	Per Cent Reduction in Germination		
8	0.5	2.0	2.5
10	0.0	1.5	1.0
12	0.0	1.0	1.5
14	1.0	0.0	0.5

Table 5.—Corrected percentages of reduction in the germination of four varieties of barley after one and two methyl bromide fumigations for 72 hours at various temperatures and dosages.

Variety of Barley	No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Lb./1000 Cu. Ft.					
			90° F.		70° F.		50° F.	
			1.0	3.0	1.5	3.0	2.0	4.0
			Per Cent Reduction in Germination					
Atlas 46	1	8	16	23	6	24	0	0
		10	20	25	17	22	1	4
		12	20	20	12	18	1	18
		14	15	19	12	17	7	18
	2	8	15	20	0	14	1	4
		10	21	22	19	20	1	5
		12	19	22	18	24	7	21
		14	18	21	17	18	9	20
California Mariout	1	8	13	13	7	19	0	0
		10	18	19	6	21	0	1
		12	12	21	10	15	1	5
		14	12	14	10	17	3	16
	2	8	20	21	12	24	0	2
		10	19	24	12	22	3	4
		12	16	19	18	21	5	10
		14	14	14	12	13	14	21
Hannchen	1	8	13	14	9	10	0	0
		10	10	11	10	15	0	1
		12	12	11	10	7	1	6
		14	9	9	13	14	6	13
	2	8	9	16	10	17	1	2
		10	14	15	11	14	2	6
		12	11	11	11	12	4	13
		14	8	12	10	15	12	14
White Smyrna	1	8	8	14	0	9	1	2
		10	11	12	6	12	1	3
		12	13	13	13	16	1	12
		14	10	12	15	14	2	8
	2	8	16	16	9	11	1	5
		10	13	19	10	13	1	9
		12	15	14	15	19	6	11
		14	10	15	11	13	11	15

Table 6.—Corrected percentages of reduction in the germination of California Mariout barley after one and two methyl bromide fumigations for 48 hours and for 24 hours at various temperatures and dosages.

No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Lb./1000 Cu. Ft.					
		90° F.		70° F.		50° F.	
		1.5	4.0	2.0	4.0	2.5	5.0
		Per Cent Reduction in Germination					
		48 hours					
1	8	2	18	3	22	0	4
	10	6	18	13	20	0	8
	12	15	22	16	15	5	17
	14	15	29	15	22	9	20
2	8	19	25	18	19	20	18
	10	18	20	21	21	17	20
	12	14	28	20	24	16	18
	14	15	28	19	25	29	27
		24 hours[a]					
1	8	4	16	2	11	0	0
	10	7	19	2	13	0	2
	12	9	16	12	20	0	5
	14	13	14	11	18	0	8
2	8	6	15	8	16	0	3
	10	12	18	13	19	0	12
	12	15	22	11	19	5	15
	14	19	22	21	21	11	16

[a] Dosage for last column at 50° F. was 4 lb. per 1000 cu. ft.

Table 7.—Corrected percentages of reduction in the germination of California Mariout barley after one and two methyl bromide fumigations for 8 hours at various temperatures and dosages.

No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosage in Lb./1000 Cu. Ft.					
		90° F.		70° F.		50° F.	
		2.0	5.0	3.0	5.0	3.0	6.0
		Per Cent Reduction in Germination					
1	8	0	2	0	3	0	0
	10	5	15	1	1	2	1
	12	7	16	3	15	0	1
	14	6	24	3	12	1	4
2	8	2	10	0	1	3	3
	10	6	14	4	13	0	1
	12	8	18	11	20	0	1
	14	15	17	15	15	0	14

Table 8.—Corrected percentages of reduction in the germination of California Mariout barley after one and two methyl bromide fumigations for 2 hours at various temperatures and dosages.

No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Lb./1000 Cu. Ft.					
		90° F.		70° F.		50° F.	
		3.0	6.0	3.0	6.0	3.0	6.0
		Per Cent Reduction in Germination					
1	8	1	1	0	0	0	0
	10	0	0	0	1	1	1
	12	2	2	1	1	0	0
	14	0	0	0	2	1	2
2	8	1	2	1	1	0	0
	10	1	2	1	1	2	0
	12	1	2	0	1	0	0
	14	1	4	0	2	0	0

ferences in the reaction of cereals to methyl bromide lie between the species. Obviously further investigation of a wide range of barley varieties was not urgent. Thus, for no other reason than being the cleanest lot of seeds, California Mariout was selected as a representative variety for use in all experiments in this study.

Moisture Content of Seeds.—Results from germination tests with barley seeds of a graded moisture range (8, 10, 12, and 14%) following fumigation with methyl bromide show a slight decrease in the percentages of germination with an increase in the moisture content (tables 5 to 8).

Period of Exposure to Fumigant.—A view of the results presented in tables 5 to 8, although not so obvious as those previously presented for wheat (Strong & Lindgren 1959), is sufficient to suggest that as periods of exposure are decreased, germination injury attributable to methyl bromide fumigation decreases.

Temperature During Fumigation.—In general, where seeds were affected by methyl bromide fumigation, increases in temperature resulted in increasing percentages of reduction in germination (tables 5 to 8). An effort was made to allow an opportunity to isolate temperature from other variables. Results are given in table 7 for fumigations with 4.0 pounds of methyl bromide per 1,000 cubic feet at each of three temperatures for 24 hours. In this particular instance, differences between 50° F. and 70° F. are greater than those between 70° F. and 90° F.

Dosage of Fumigant.—Eradication programs require somewhat higher than normal dosages of fumigant so two dosage levels, one low and one high, were considered for each set of conditions found in this study. Paired comparisons may be made between dosage levels from the results presented in tables 5 to 8 by horizontal examination of the data. However, the influence of dosage is best illustrated with results from one fumigation as seen in tables 6 and 7.

Repeat Fumigation.—Seed sanitation usually requires more than one fumigation to prevent injury from insect pests. Thus, the effect of repeat fumigation on the germination of seeds was an important factor to consider. In some cases increased injury to germination may be observed from two fumigations, but, in general, two fumigations with methyl bromide did not have an extreme effect on the germination of barley (tables 5 to 8).

Postfumigation Storage.—A second series of germination tests was made after postfumigation storage of seeds for 84 days (12 weeks) at 50° F. Significant differences were not observed in the percentages of reduction in germination between tests made immediately (within 5 days) after fumigation and those made 84 days later. Results from the latter tests are not presented as they would merely duplicate results given in tables 5 to 8. These results concur with those of Cobb (1958) and agree with his conclusion that fumigation injury occurred while seeds were in contact with the fumigant, that fumigation injury was irreversible, and seeds uninjured during fumigation do not show injury later.

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Toxicity of Several Residual-Type Insecticides to Selected Western Bark Beetles[1]

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ABSTRACT

The relative potency of several residual-type insecticides to the adult of selected western species of bark beetles was determined by topical application. The decreasing order of relative potency (95% confidence) for four insects was as follows:

The western pine beetle (*Dendroctonus brevicomis* Lec.)—endrin, isodrin, EPN, and lindane (endrin and isodrin>lindane)>dieldrin>heptachlor, dinitrocresol, and DDT.

The mountain pine beetle (*Dendroctonus monticolae* Hopk.)—lindane, EPN, and endrin>dieldrin and heptachlor>dinitrocresol>DDT.

The fir engraver (*Scolytus ventralis* Lec.)—isodrin, endrin, dieldrin, and lindane (isodrin and endrin>lindane)>EPN>DDT>dinitrocresol.

The California flatheaded borer (*Melanophila californica* Van D.)—lindane, isodrin, dieldrin, and DDT (lindane and isodrin>DDT).

An analysis of toxicity to the sexes of *D. brevicomis* and *D. monticolae* showed only two insecticides significantly more toxic to one of the sexes: heptachlor was 3.31±0.66 times more toxic to the male *D. monticolae;* dinitrocresol was 4.02±0.46 times more toxic to the female *D. brevicomis.*

A less costly, more reliable method for chemical control of bark beetles infesting forest trees is needed. The chief current method uses penetrating oil sprays. This is a costly procedure because the sprays are applied liberally to the bark of infested trees that first are felled, limbed, and bucked. The recommended dose for a 30-inch infested ponderosa pine is 25 gallons of spray composed of ethylene dibromide and diesel oil (Downing 1954). Furthermore, penetration sometimes is incomplete when the bark is much thicker than 1 or 2 inches or when the moisture content of bark tissue is high enough to retard the movement of the spray oil.

Investigations by Moore (1953) have suggested that the residual-type, chlorinated hydrocarbon insecticides may be more reliable and less costly than penetrating oil sprays. Therefore the Experiment Station has started a research program seeking to develop bark beetle control with toxic, long lasting deposits on the bark surface of the host tree.

Such deposits of long lasting toxicity not only offer a way of killing bark beetles emerging from infested trees, but also promise to protect uninfested trees from attack. Protection of a tree is not possible with penetrating oil sprays, which have short residual life and are difficult to apply to standing trees. Residual sprays on the bark surface may also protect felled timber subject to attack by bark beetles of the genus *Ips.*

A first step in the Station's research program was to screen selected residual-type insecticides (Moore 1957) by means of topical application of acetone solutions to the thoracic sterna of the western pine beetle (*Dendroctonus brevicomis* Lec.) and the California five-spined engraver (*Ips confusus* (Lec.)). The studies reported here are an extension of screening tests begun by Moore. Field work was conducted at the Miami Field Laboratory maintained near Oakhurst, California, by the Forest Service. Much credit is due W. D. Bedard for his capable assistance.

MATERIALS AND METHODS.—*Test Insects.*—All test insects were collected in the vicinity of the Miami Field Laboratory except the California flatheaded borer (*Melanophila californica*) which was obtained from the San Jacinto Mountains of southern California. The beetle species tested and the hosts from which they were reared are:

Insect	*Host*
western pine beetle, *Dendroctonus brevicomis* Lec.	ponderosa pine, *Pinus ponderosa* Laws.
mountain pine beetle, *Dendroctonus monticolae* Hopk.	lodgepole pine, *Pinus contorta* Dougl.
fir engraver, *Scolytus ventralis* Lec.	white fir, *Abies concolor* (Gord. & Glend.) Lindl.
California flatheaded borer, *Melanophila californica* Van D.	Jeffrey pine, *Pinus jeffreyi* Grev. & Balf.

Brood of *D. brevicomis, D. monticolae,* and *S. ventralis* was collected by cutting the boles of infested trees in 1.5 to 2-foot lengths. Brood of *M. californica* was obtained by collecting the bark only. The infested material was refrigerated at about 47 degrees F. to prevent emergence and then transferred piecemeal to outdoor, wire screen rearing cages at intervals that furnished a continuous supply of freshly emerged adults for testing.

Each beetle, as it emerged, was collected singly in a #000 gelatin capsule to prevent the insects from injuring themselves as they do when confined in close quarters.

Uniformity in the age of test insects was maintained by allowing no more than 6 hours to lapse between emergence and treatment by topical application. All treated and untreated samples, therefore, were composed of adults that had emerged between 0 and 6 hours before. This variation in age had no influence on mortality in the controls.

Insecticides.—The following is a list of the insecticides[3] chosen for testing, and the purity of the product from which solutions were made:

Insecticide	*Purity*
DDT	c. 100% (recrystallized from tech. grade) m.p. 108.5–109 C.
Dieldrin	85%
Dinitrocresol	Tech. grade
Endrin	93.4%

(*Continued on next page*)

[1] Accepted for publication October 13, 1958.

[2] Maintained at Berkeley, California, in cooperation with the University of California.

[3] These are named according to the approved common names of insecticides published by the Committee on Insecticide Terminology of the Entomological Society of America—Jour. Econ. Ent. 50 (2): 226–8.

Insecticide	*Purity*
EPN	Tech. grade
Heptachlor	c. 100% (recrystallized from tech. grade) m.p. 92–96 C.
Isodrin	91.3%
Lindane	c. 100%

All insecticides were tested in an acetone solution.[4] Each solution was prepared in concentrated form and diluted as needed for testing. The dilutions were held in dropping bottles with frosted glass stoppers and refrigerated to reduce loss by evaporation. No measurable evaporation took place under refrigeration during a period of 2 weeks, and all dilutions were discarded after 10 to 14 days' use.

Treating Apparatus.—Topical application was made with a modified micrometer and a 0.25-ml. tuberculin syringe. The syringe was attached in direct line with the micrometer spindle and served as a reservoir for the test solution. To permit application of small amounts of the solution, the syringe was fitted at its apex with a 26-gauge hypodermic needle. The needle was bent at 90 degrees to facilitate the transfer of the solution from the needle tip to the body of the insect.

Procedure.—Each insect was handled and treated individually. It was removed from the gelatin capsule in which it was collected, and placed on its dorsum on the glass stage of a binocular dissecting scope. On glass, the insect was unable to right itself. The required volume of test solution was produced as a small drop at the needle tip of the treating apparatus and brought in contact with the ventral surface of the thorax. As the drop of solution touched the insect's body, it broke cleanly from the needle tip and spread rapidly over the thoracic sternites. After the insects were treated they were replaced in gelatin capsules and held in open Petri dishes at a temperature of 62 to 68° F. and a relative humidity of 60 to 70%. Mortality counts were made 72 hours after treatment.

All four species varied considerably in weight. *D. brevicomis* ranged from 1 to 10 mg.; *D. monticolae:* 6 to 20 mg.; *S. ventralis:* 2 to 10 mg., and *M. californica:* 15 to 55 mg. Application of an equal dose to each insect was not advisable because of this wide variation in weights, so the insect was given a volumetric dose proportional to its body weight. This was done by setting up weight classes of the insects in Riker mounts. Before treatment, the insect was classed visually with those in the Riker mount to determine the proper dose to administer. All test beetles were treated with 0.16 lambda (1 lambda = 1/1000 ml.) of test solution for each mg. of body weight, the amount delivered by a turn of the micrometer spindle through two divisions.

Before a beetle was treated, the micrometer spindle was turned one complete revolution (50 divisions) and the drop of solution produced at the needle tip was removed with cotton. This procedure was adopted because the solvent evaporates from the needle passage between treatments, and the dose administered would vary somewhat if precautions were not taken to bring the test solution back to the needle opening before each treatment. When treatment with a test solution was completed, the syringe and needle were cleaned thoroughly in acetone to prevent contamination in subsequent tests. Solutions of a given series were tested in increasing order of concentration to help minimize the chances of contamination.

Regression lines of mortality over dosage were made with mortality data from a series of usually between 8 to 10 different concentrations of the candidate insecticide. Each concentration was tested with a sample of 20 insects, sometimes replicated 2 to 3 times. Samples of 20 insects were set aside, untreated, to serve as controls. Insects were also treated with acetone alone, although there was no significant difference in mortality between the acetone and the untreated controls. Mortality data was rectified by the probit method (Finney 1952).

RESULTS.—Relative potency of all test insecticides was determined with the assumption, based on chi-square tests (table 1), that a parallel relationship existed between each candidate insecticide and the standard, DDT. Figure 1 shows the dosage-mortality relationships for each of the four experimental insects.

The order of potency of the candidate insecticides, allowed by the 95% fiducial limits of relative potency (table 2), was as follows:

D. brevicomis

endrin, isodrin, EPN, lindane > dieldrin > heptachlor > dinitrocresol > DDT

D. monticolae[5]

lindane, EPN, endrin > dieldrin, heptachlor > dinitrocresol > DDT

S. ventralis

isodrin, endrin, dieldrin, lindane > EPN > DDT > dinitrocresol

M. californica

lindane, isodrin, dieldrin, DDT

This grouping of the insecticides brings out several points of interest.

1. The insecticide of greatest potential toxicity to *D. brevicomis* may be either endrin or isodrin. Both were about 11 times more potent than DDT.

2. The potentially most potent insecticide to *D. monticolae* may be either lindane, EPN, or endrin. Isodrin, also very potent, was too variable to permit predictions regarding its relative potency. Lindane is more probably the most potent insecticide—about 21 times the potency of DDT. EPN and endrin were both about 14 times more potent than DDT.

3. Either isodrin or endrin may be the potentially most potent insecticide to *S. ventralis*—isodrin at about 29 and endrin at about 20 times the potency of DDT.

4. Lindane or isodrin may be the potentially most po-

[4] Solutions were based upon the active principle for all insecticides except EPN and dinitrocresol.

[5] Data for isodrin in tests with *D. monticolae* were too variable to permit a worthwhile estimate of its potency in relation to the other candidate insecticides.

Table 1.—Parallelism of the mortality response to selected residual-type insecticides.

Insecticide	Chi-Square Value of Test for Parallelism[a]	Approximate Probability of a Larger Chi-Square Value
Dendroctonus brevicomis		
Lindane	0.39	0.50
EPN	0.79	.40
Isodrin	1.14	.30
Heptachlor	2.15	.15
Dieldrin	2.69	.10
Dinitrocresol	2.81	.10
Endrin	3.54	.06
Dendroctonus monticolae		
Lindane	0.02	0.90
EPN	0.03	.90
Isodrin	0.07	.80
Endrin	0.70	.40
Dinitrocresol	0.95	.30
Heptachlor	1.35	.25
Dieldrin	3.32	.07
Scolytus ventralis		
Dinitrocresol	0.01	0.90
Dieldrin	0.53	.50
Endrin	0.79	.35
Lindane	0.93	.30
EPN	1.41	.25
Isodrin	3.15	.08
Melanophila californica		
Isodrin	0.04	0.85
Dieldrin	0.61	.45
Lindane	0.98	.30

[a] One degree of freedom.

Table 2.—Relative potency of selected residual-type insecticides.

Insecticide	Relative Potency and Standard Error	Fiducial Limits (95% Probability)
Dendroctonus brevicomis		
Endrin	11.32±1.03	9.30–13.34
Isodrin	11.10±1.01	9.12–13.08
EPN	8.59±0.94	6.79–10.43
Lindane	7.32±0.85	5.65– 8.99
Dieldrin	2.40±0.30	1.81– 2.99
Heptachlor	1.21±0.17	0.88– 1.54
Dinitrocresol	1.00±0.12	0.77– 1.23
DDT	1.00 (standard)	
Dendroctonus monticolae		
Lindane	21.09±2.75	15.70–26.48
Isodrin	18.01±9.48	too variable
EPN	14.86±1.81	11.31–18.41
Endrin	14.57±1.77	11.10–18.04
Dieldrin	7.47±1.12	5.28– 9.66
Heptachlor	6.25±0.92	4.45– 8.05
Dinitrocresol	3.11±0.37	2.39– 3.83
DDT	1.00 (standard)	
Scolytus ventralis[a]		
Isodrin	29.07±7.42	14.53–43.61
Endrin	20.41±4.43	11.73–29.09
Dieldrin	10.51±2.67	5.28–15.74
Lindane	8.12±1.61	4.97–11.27
EPN	2.34±0.45	1.46– 3.22
DDT	1.00 (standard)	
Dinitrocresol	0.49±0.08	0.33– 0.65
Melanophila californica[a]		
Lindane	12.53±5.10	2.53–22.53
Isodrin	8.92±2.89	3.26–14.58
Dieldrin	2.45±0.74	1.00– 3.90
DDT	1.00 (standard)	

[a] Mortality data from tests with *S. ventralis* and *M. californica* were more variable than data on the other two species. With *S. ventralis*, this can be imputed largely to the unusually high check mortality. The average was 43% and the range was 10% to 80%. High variability with *M. californica* was probably due to the wide range in tolerance which this insect showed toward all the insecticides tested on it. As a result, a given sample contained individuals of vastly different tolerance thresholds.

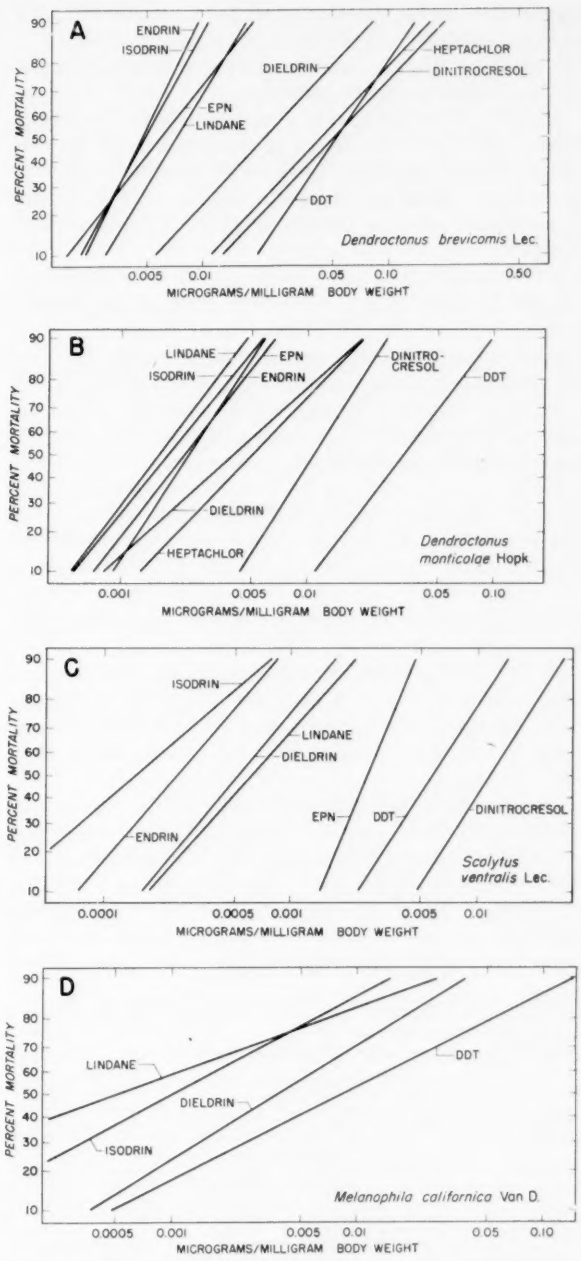

FIG. 1.—Dosage-mortality relationships obtained by topical application of the test insecticides to the thoracic sterna of adult beetles. (A) *Dendroctonus brevicomis* Lec.; (B) *Dendroctonus monticolae* Hopk.; (C) *Scolytus ventralis* Lec.; (D) *Melanophila californica* Van D.

tent insecticide to *M. californica*. Lindane was about 12 and isodrin was about 9 times the potency of DDT.

5. Endrin, isodrin, and lindane were generally superior in potency with all test beetles.

6. EPN varied more widely in its effect than any of the other insecticides. No other test insecticide could be said to be more potent than EPN to *D. monticolae* at 95% confidence. However, isodrin, endrin, lindane, and dieldrin were all more potent than EPN in tests with *S. ventralis*.

7. Dieldrin was usually more potent than DDT although its toxicity was generally excelled by isodrin, endrin, lindane, and EPN. Only once did dieldrin show greater potency than any one of these four toxicants, and that was in tests with *S. ventralis:* dieldrin > EPN > DDT.

8. Dinitrocresol and heptachlor were generally the least potent candidates (excluding DDT). They were the least potent insecticides to *D. brevicomis:* inseparable from each other and from DDT at 95% confidence. Only in tests with *D. monticolae* did heptachlor approach the potency of the more toxic candidates. Heptachlor and dieldrin could not be separated with 95% confidence in these tests. DDT was the only insecticide that was excelled in potency by dinitrocresol and heptachlor, and this occurred only in tests with *D. monticolae*.

9. DDT, in general, was the least potent of all the candidate insecticides tested. Only once did DDT surpass another insecticide in potency, and this occurred in tests with *S. ventralis*, where DDT was more potent than dinitrocresol.

10. A generalized order of toxicity of the insecticides tested on *D. brevicomis*, *D. monticolae*, *S. ventralis*, and *M. californica* is as follows: isodrin, endrin, lindane, and EPN > dieldrin > heptachlor and dinitrocresol > DDT.

Tolerance Differences Between Test Species.—Judging from the amount of insecticide needed to get 50% and 90% kills (table 3), the relative tolerance of the four test insect species was much the same at both the LD_{50} and LD_{90} levels of kill (table 4). The one exception was *M. californica*, which showed a major shift in tolerance from LD_{50} to LD_{90}. A generalized order of increasing tolerance at LD_{50} is: *S. ventralis* < *M. californica* < *D. monticolae* < *D. brevicomis;* at LD_{90}: *S. ventralis* < *D. monticolae* < *D. brevicomis* = *M. californica*.

Table 3.—Toxicity of selected residual-type insecticides by actual dosage values.

Insecticide	LD_{50} and Standard Error (μG./Mg. Body Weight)	Insecticide	LD_{90} and Standard Error (μG./Mg. Body Weight)
	Dendroctonus brevicomis		
Endrin	0.0048 ±0.0003	Endrin	0.0096 ±0.0009
Isodrin	0.0049 ±0.0003	Isodrin	0.0109 ±0.0011
EPN	0.0060 ±0.0005	Lindane	0.0174 ±0.0024
Lindane	0.0073 ±0.0007	EPN	0.0191 ±0.0028
Dieldrin	0.0218 ±0.0023	Dieldrin	0.0832 ±0.0173
Heptachlor	0.0437 ±0.0061	DDT	0.1405 ±0.0200
Dinitrocresol	0.0514 ±0.0047	Heptachlor	0.1698 ±0.0519
DDT	0.0530 ±0.0037	Dinitrocresol	0.2013 ±0.0409
	Dendroctonus monticolae		
Lindane	0.0016 ±0.0002	Lindane	0.0047 ±0.0007
Isodrin	0.0018 ±0.0004	Isodrin	0.0057 ±0.0014
EPN	0.0022 ±0.0002	Endrin	0.0058 ±0.0007
Endrin	0.0023 ±0.0002	EPN	0.0067 ±0.0008
Dieldrin	0.0040 ±0.0004	Dieldrin	0.0195 ±0.0035
Heptachlor	0.0051 ±0.0006	Heptachlor	0.0200 ±0.0041
Dinitrocresol	0.0109 ±0.0008	Dinitrocresol	0.0263 ±0.0034
DDT	0.0330 ±0.0031	DDT	0.0962 ±0.0159
	Scolytus ventralis		
Isodrin	0.00015±0.00003	Isodrin	0.00079±0.00020
Endrin	0.00025±0.00005	Endrin	0.00085±0.00017
Dieldrin	0.00053±0.00011	Dieldrin	0.00172±0.00067
Lindane	0.00063±0.00011	Lindane	0.00224±0.00054
EPN	0.00260±0.00026	EPN	0.00466±0.00087
DDT	0.00585±0.00092	DDT	0.01459±0.00270
Dinitrocresol	0.01194±0.00123	Dinitrocresol	0.02908±0.00524
	Melanophila californica		
Lindane	0.0005 ±0.0002	Isodrin	0.0144 ±0.0049
Isodrin	0.0010 ±0.0002	Lindane	0.0260 ±0.0223
Dieldrin	0.0037 ±0.0007	Dieldrin	0.0373 ±0.0147
DDT	0.0082 ±0.0022	DDT	0.1422 ±0.0743

Table 4.—Test insects arranged in increasing order of tolerance to each candidate insecticide based on actual amounts of insecticide needed for 50% and 90% kills.[a]

Insecticide	Insect Species[b] at LD_{50}	at LD_{90}
DDT	S.v. = M.c. < D.m. < D.b.	S.v. < D.m. < D.b. = M.c.
Isodrin	S.v. < M.c. < D.m. < D.b.	S.v. < D.m. < D.b. = M.c.
Lindane	S.v. = M.c. < D.m. < D.b.	S.v. < D.m. < D.b. = M.c.
Dieldrin	S.v. < M.c. = D.m. < D.b.	S.v. < D.m. = M.c. < D.b.
Endrin	S.v. < D.m. < D.b.	S.v. < D.m. < D.b.
EPN	S.v. = D.m. < D.b.	S.v. < D.m. < D.b.
Dinitrocresol	S.v. = D.m. < D.b.	S.v. = D.m. < D.b.
Heptachlor	D.m. < D.b.	D.m. < D.b.

[a] The symbols = (equal to) and < (less than) were used when standard errors did and did not overlap, respectively.

[b] S.v. is *Scolytus ventralis*; M.c. is *Melanophila californica*; D.m. is *Dendroctonus monticolae*; D.b. is *Dendroctonus brevicomis*.

Toxicity by Sex.—The toxicity of the candidate insecticides to the sexes of *D. brevicomis* and *D. monticolae* was determined. Mortality of the males of both species was usually higher but differences could not be shown statistically except in tests with heptachlor on *D. monticolae*. This insecticide was 3.31 ± 0.66 (± standard error) times more potent to the male. Only one insecticide showed greater toxicity to the female sex. Dinitrocresol was 4.02 ± 0.46 (± standard error) times more potent to the female of *D. brevicomis*.

When there is a major difference in toxicity by sex as there is in these two examples, a more realistic measure of relative potency can be had by including only the more easily killed sex in the analysis. It may well be as effective to control an insect by killing one sex as by killing both.

The relative potency of heptachlor, to *D. monticolae*, is much greater when the males alone are considered since

Table 5.—The magnitude of differences in tolerance to the residual-type insecticides between insect species at LD_{90}.[a]

Insecticide	*D.b.* vs *D.m.*[b]	Insecticide	*D.b.* vs. *S.v.*	Insecticide	*D.m.* vs. *S.v.*
Heptachlor	8.5	Dieldrin	48.8	Dieldrin	11.3
Dinitrocresol	7.6	Isodrin	13.8	Isodrin	7.2
Dieldrin	4.3	Endrin	11.3	Endrin	6.8
Lindane	3.7	DDT	9.6	DDT	6.6
EPN	2.8	Lindane	7.8	Lindane	2.1
Isodrin	1.9	Dinitrocresol	6.9	EPN	1.4
Endrin	1.6	EPN	4.1	Dinitrocresol	0.0
DDT	1.5				

Insecticide	*M.c.* vs. *D.m.*	Insecticide	*M.c.* vs. *S.v.*
Lindane	3.1	Dieldrin	21.7
Isodrin	2.5	Isodrin	18.2
Dieldrin	1.9	DDT	9.7
DDT	1.5	Lindane	6.4

[a] The numerals denote the number of times the first insect heading the column exceeds the second in tolerance.

[b] *D.b.* is *Dendroctonus brevicomis*; *D.m.* is *Dendroctonus monticolae*; *S.v.* is *Scolytus ventralis*; *M.c.* is *Melanophila californica*.

the male sex was more easily killed by heptachlor. The following shows the improvement of the relative potency of heptachlor when only the males were included in the analysis:

	Relative potency and standard error at LD_{90}
Heptachlor (males and females)	6.25±0.92
Heptachlor (males only)	13.02±2.33

Considering only the males in the analysis of relative potency of heptachlor, the potency of the candidate insecticides to *D. monticolae*, in decreasing order, changes from: Lindane, EPN, and endrin > dieldrin and heptachlor > dinitrocresol > DDT, to: Lindane, EPN, endrin, and heptachlor > dieldrin > dinitrocresol > DDT. Heptachlor, therefore, is one of the more promising residual insecticides tested if its superior effect on the male sex is taken into account.

When the relative potency of dinitrocresol to *D. brevicomis* is calculated by excluding the males, the improvement in its relative potency is as follows:

	Relative potency and standard error at LD_{90}
Dinitrocresol (males and females)	1.00±0.12
Dinitrocresol (females only)	2.91±0.48

By including only the females treated with dinitrocresol in the analysis of relative potency, the order of decreasing potency of the candidate insecticides to *D. brevicomis* changes from: endrin, isodrin, EPN, and lindane > dieldrin > heptachlor, dinitrocresol, and DDT, to: endrin, isodrin, EPN, and lindane > dinitrocresol and dieldrin > heptachlor and DDT. The relative potency of dinitrocresol also shows an apparent improvement when its greater toxicity to the female is taken into account. The level of potency of dinitrocresol, to *D. brevicomis*, can be considered greater than DDT and heptachlor and equal to dieldrin.

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Partial Immunity of *Laphygma exigua* (Hübner) to the Parasite *Hyposoter exiguae*[1] (Viereck)[2]

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ABSTRACT

Larvae of the noctuid *Laphygma exigua* (Hübner) have the ability to phagocytose and encapsulate eggs of the ichneumonid *Hyposoter exiguae* (Viereck). A high percentage of the *L. exigua* larvae containing single parasite eggs escape fatal parasitization through this phagocytic action. However, in superparasitized hosts immunity generally breaks down because of the inability of the host larvae to encapsulate supernumerary parasite eggs. Superparasitism, therefore, in the *L. exigua-H. exiguae* relationship is advantageous to the parasite.

The Nearctic ichneumonid *Hyposoter exiguae* (Viereck) is a solitary endoparasite of larvae of a number of lepidopterous species, most of which belong to the family Noctuidae. In California it is a major parasite of the tomato fruitworm (*Heliothis zea* (Boddie)) (Michelbacher & Essig 1938, Wilcox *et al.* 1956), and also attacks the beet armyworm (*Laphygma exigua* (Hbn.)), the western yellow-striped armyworm (*Prodenia praefica* Grote), the variegated cutworm (*Peridroma margaritosa* (Haw.)), and the cabbage looper (*Trichoplusia ni* (Hbn.)).

Recent investigations of the biology of this important parasite have brought to light information on the relative suitability of certain host species to it. One of the most interesting findings concerns the partial immunity of *Laphygma exigua* to *Hyposoter exiguae*. The nature of this immunity and the mechanism whereby it is prevented from attaining even higher proportions are discussed in this paper.

EXPERIMENTAL METHODS.—Stocks of *Hyposoter exiguae* were maintained in the laboratory through continuous rearing on larvae of *Peridroma margaritosa*. The *Laphygma exigua* larvae were obtained from egg masses deposited by moths previously collected at lights in Riverside, California, and were reared on alfalfa bouquets in half-pint ice-cream cartons closed at the open ends with petri dishes. The laboratory temperature ranged between 72° and 76° F. and relative humidity from 30% to 50%.

Late first- and second-instar larvae were utilized for parasite oviposition, as previous study had revealed that these were the optimum stages for *Hyposoter exiguae* oviposition and for the subsequent successful development of the parasite larvae.

Parasitization was effected in two ways. In the first technique *Laphygma exigua* larvae were placed in vials with single *Hyposoter exiguae* females and removed as soon as oviposition was observed. The stung larvae were then held for 48 hours before being examined for parasites. Where superparasitization was desired, previously

[1] Hymenoptera: Ichneumonidae.

[2] Accepted for publication October 15, 1958.

Table 1.—Reaction of *Laphygma exigua* larvae to eggs of *Hyposoter exiguae*.

Type of Parasitism	Number of Parasitized Larvae in Sample	Number of Larvae with Completely Phagocytozed Eggs	Per Cent Complete Phagocytosis	No. Larvae with Partially Phagocytozed Eggs	Per Cent Partial Phagocytosis	No. Larvae with Eggs Escaping Complete Phagocytosis	Per Cent Larvae with Eggs Escaping Complete Phagocytosis
Solitary	50	46	92.0	0	0.0	4	8.0
Superparasitism	30	1	3.3	29	96.7	29	96.7

parasitized larvae were exposed 2 hours after being initially attacked and removed when the second oviposition was observed.

In the second parasitization technique 15 to 25 host larvae were exposed to a single *Hyposoter exiguae* female for 48 hours. They were held for an additional 24 hours after the parasite had been removed before being examined for evidence of parasitism. The fate of the parasite eggs and larvae was ascertained by dissecting the larvae in saline solution in a stender dish under a binocular microscope.

Nature of Immunity.—Dissections revealed that the immunity effected by *Laphygma exigua* resulted from the encapsulation of the egg of *Hyposoter exiguae* by blood cells of the host, a phenomenon commonly referred to as phagocytosis. Phagocytosis of parasite eggs and larvae in nonadapted hosts is rather common (Bess 1939, Boese 1936, Eckstein 1931, Salt 1955, 1956) and frequently results in total immunity of a host species to a particular parasite. In normal or adapted hosts this reaction does not usually occur (Timberlake 1912). An exception appears to have taken place in the relationship between the larch sawfly (*Pristiphora erichsonii* (Htg.)) and its parasite *Mesoleius tenthredinis* Morley. *P. erichsonii* has become immune to the parasite in Manitoba and Saskatchewan, while remaining susceptible in British Columbia (Muldrew 1953).

The extent of encapsulation of *Hyposoter exiguae* eggs by the phagocytes (blood cells) of *Laphygma exigua*, as revealed by dissections, was considerable, as shown in table 1. However, a high degree of immunity occurred only in larvae containing single parasite eggs. Dissections revealed that where superparasitism occurred the immunity was largely overcome. Thus, complete phagocytosis took place in 92.0% of the larvae containing single eggs, whereas in superparasitized individuals complete phagocytosis occurred in only 3.3% of the larvae.

Of the 30 superparasitized larvae listed in table 1, five contained more than two eggs. These five larvae all encapsulated single eggs and the remaining eggs survived and hatched. However, the supernumerary parasite larvae apparently were killed by internecine action soon after hatching, leaving but a single living larva in each host. The small, dead larvae were eventually encapsulated by blood cells of *Laphygma exigua*. This suggests that *L. exigua* larvae may contain in their blood two distinct types of phagocytic cells, one that attacks and destroys living parasite eggs and one that reacts to and encapsulates inanimate foreign bodies. On the other hand, the ability of the *L. exigua* larvae to encapsulate the dead parasite larvae may result from the regeneration of phagocytic cells after a time lapse following encapsulation of the initially deposited parasite egg.

Immunity in Field-Collected *Laphygma exigua*.—In addition to the immunity data obtained in the laboratory, limited observations on this phenomenon were also made in field-collected larvae. *L. exigua* larvae collected from alfalfa at Borrego, San Diego County, California, were utilized in this study, which was somewhat complicated by multiple parasitism (*i.e.*, simultaneous parasitization by more than one parasite species). Rearings from these larvae show the complex of parasites to have been comprised of three species: *Rogas* sp. (Braconidae), *Hyposoter exiguae*, and a tachinid. From 50 larvae in the Borrego sample that were dissected, 10 were found to contain eggs and larvae of *H. exiguae*. Four of the 10 larvae contained only single eggs of *H. exiguae*, all of which were phagocytozed. Five of the remaining six larvae were superparasitized, each containing a living parasite larva as well as phagocytozed eggs. The 10th *L. exigua* larva contained a single living parasite larva.

Discussion.—The data presented above rather clearly indicate that *Laphygma exigua* possesses a partial immunity to parasitism by the ichneumonid *Hyposoter exiguae*. This immunity, which is effected by phagocytes, is very high in unsuperparasitized larvae but breaks down in most larvae containing more than one egg.

The partial immunity of *Laphygma exigua* to *Hyposoter exiguae* indicates that this noctuid is not one of the most favorable hosts of the parasite. Perhaps the most interesting aspect of the relationship is the role of superparasitism in the breakdown of immunity. It is generally assumed that superparasitism is undesirable, as it contributes to parasite egg wastage (Fiske 1910). However, in the present case superparasitism appears to be advantageous because it enables *H. exiguae* to parasitize a higher percentage of the host population than it would otherwise.

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Insecticidal Control of the Lesser Cornstalk Borer on Peanuts[1]

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ABSTRACT

This study was conducted to develop an economical control for the lesser cornstalk borer (*Elasmopalpus lignosellus* (Zeller)) on peanuts.

To determine the effects of varying volumes of spray mixture on control, a constant amount of endrin was applied in 20, 40 and 86 gallons of spray mixture per acre. A highly significant difference was obtained between the treatments and the check in both the per cent of damaged peanuts and in the yield. No differences were attributable to variations in the volume of spray mixture applied per acre.

To determine the dates at which insecticides should be applied to develop a satisfactory spray schedule, DDT was applied in combinations of single and double applications during the periods of moth flight in June, July and August. The per cent of injury was reduced significantly by the treatments. No significant differences in yield were observed between treatments.

In an experiment in which insecticides were applied at 7-, 14-, and 21-day intervals, injury was reduced significantly by each of the treatments in comparison with the check. No differences were observed between treatments. The data on yields show no significant differences between the various treatments or between the treatments and the check.

During recent years, increasingly severe damage to peanuts by the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), has occurred. A research program was initiated in 1954 at Stephenville, Texas, to develop a control for this pest.

Seasonal life history and control studies by Sanchez (unpublished data) in 1955 and 1956 provided the basis for research which demonstrated that effective control could be obtained with insecticides.

EXPERIMENTAL METHODS.—During 1957, field experiments were conducted in which insecticides were applied with a 2-row tractor-mounted spray machine capable of delivering spray materials at various gallonages and pressures. The spray was applied from each side of the row to insure coverage of the stems and the ground at the base of the plants. In previous tests, control was not obtained by vertical spraying of the tops of the plants.

The effectiveness of insecticide treatments was evaluated by counting the peanuts on each plant in a sample of 10 plants selected at random from each plot and determining the percentage injured by the lesser cornstalk borer. This insect chews a small hole in the shell and feeds on the kernels. Strands of silk adhere to soil particles and plant parts in the feeding area. Populations of other insects which attack peanuts in a similar manner have been negligible in central Texas where this study was performed.

Records of individual plot yields were obtained. This was accomplished by harvesting the plants in 25 feet of row in each plot and weighing the unshelled peanuts.

Volume of Spray.—An experiment was conducted to determine the effect on lesser cornstalk borer injury by varying the volume of spray mixture applied. Endrin was applied in an emulsion spray at the rate of 0.4 pound active ingredient in 20, 40 and 86 gallons of spray mixture per acre. Variations in spray volume were effected by altering the tractor speed. Applications were made on July 3, August 2 and August 30, 1957. These dates were selected to coincide with the appearance of successive generations of the moth as determined by previous research (Sanchez, unpublished data). All insecticides were applied at a pressure of 50 p.s.i.

Each plot was eight rows wide and 34 feet long. The outside rows were guard rows, the two middle rows were used to obtain yield records and the remaining four rows were sampled periodically to evaluate the effectiveness of the treatments. Sample plants were obtained on August 15, and immediately preceding harvest on October 19, 1957.

A highly significant difference in injury and yield was obtained between the spray treatments and the check, but there were no significant differences due to variations in the volume of spray mixture applied per acre. Sample and yield data are presented in table 1.

Timing of Spray Applications.—A second experiment was conducted to determine the dates on which spray applications should be made to provide the most effective

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Table 1.—The per cent injury on two sampling dates and yield of peanuts following the application of endrin in varying gallonages of spray mixture for control of the lesser cornstalk borer.

Treatment (Gals./A.)	Per Cent Damaged		Lbs. Unshelled Peanuts/25 Ft. Row
	August 15	October 19	
20	3.5	2.0	1.8
40	2.0	2.7	1.7
86	1.8	5.4	2.1
Check	18.8	11.5	.9
L.S.D. at 5% level	5.76	3.93	.50

practical control of the lesser cornstalk borer. DDT emulsifiable concentrate was applied at 1.5 pounds active ingredient in 100 gallons of spray mixture per acre at a pressure of 100 p.s.i. Treatments were applied at approximately monthly intervals to coincide with the appearance of successive generations of moths in the area.

Each plot was six rows wide and 50 feet long. The outside rows were guard rows, the two middle rows were used to obtain yield records, and the remaining two rows were sampled periodically to evaluate the effectiveness of the treatments. Seven treatments were included in each of four replications. The experiment was designed as a randomized block.

Ten sample plants were taken from each plot on October 3, 1957, and inspected for injury by the lesser cornstalk borer. The dates on which the applications were made together with the per cent injury in each treatment are presented in table 2. Analysis disclosed a significant

Table 2.—Per cent of damaged peanuts on October 3 following the varying of dates of application of DDT.

Time of Application	Per Cent Injury
7/1	3.1
7/29	5.2
8/27	2.7
7/1, 7/29	3.2
7/1, 8/27	1.2
7/29, 8/27	2.8
Check	8.9
L.S.D. at 5% level	3.53

Table 3.—Per cent of damaged peanuts following applications of insecticides at varying intervals.

Interval between Treatments	Per Cent Damaged Peanuts	
	August 23	October 2
7-Day	2.8	1.9
14-Day	5.5	2.3
21-Day	5.8	1.7
Check	29.3	10.4
L.S.D. at 5% level	8.07	4.74

difference in injury between insecticidal treatments and the check. No significant differences in yields were obtained. This probably was due either to variations in the stand of plants in the plots or to the fact that the infestation by the lesser cornstalk borer was very light.

Another experiment was initiated to determine whether spray applications at more frequent intervals during the periods of moth flight of each generation would result in more effective control. The intervals between applications of insecticide for the different schedules were established at 7, 14, and 21 days. The insecticidal treatments and a check were randomized and replicated four times.

The insecticide was applied to plots 30 feet long and six rows wide. A combination of endrin and DDT was applied at the rate of 0.4 and 1.5 pounds of active ingredient per acre, respectively, in an emulsion spray. Applications were made at a pressure of 100 p.s.i, and at the rate of 100 gallons of spray mixture per acre.

The 21-day interval treatment was made on July 25 and on August 19, 1957. The 14-day interval treatment was made July 29 and August 12, 1957. The 7-day interval treatment was made July 29, August 5, August 12 and August 19, 1957. Ten sample plants were obtained from each plot on August 23 and October 2, 1957. There was a difference, significant at the 1% level, between these treatments and the check on both sampling dates, but there were no significant differences between the treatments (table 3). Records on the yields were obtained, but there was no significant difference in the yields between the treatments and the check.

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The Evaluation of Insecticides for the Control of *Tendipes plumosus* (Linnaeus)[1]

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ABSTRACT

In laboratory tests, 16 commercially available organophosphate insecticides were screened to determine their relative toxicity to larvae of *Tendipes plumosus* (Linnaeus) and to the fathead minnow, *Pimephales promelas* Rafinesque. DDVP, Dipterex, EPN, Guthion, malathion, and Phosdrin all showed considerable toxicity to the larvae at concentrations of 0.1 pounds or less per acre of the technical material incorporated into granules. Of these six insecticides, DDVP at 50 pounds per acre, Dipterex at 100 pounds per acre, and malathion at 20 pounds per acre showed no toxicity to the minnows in replicated tests. These same three insecticides, in replicated tests, all produced 97% mortality of *T. plumosus* larvae within 7 days at concentrations of 0.75 pounds per acre and Dipterex produced 97% mortality at 0.05 pounds per acre.

Serious outbreaks of the midge, *Tendipes plumosus* (Linnaeus), have plagued residents and industries in the Lake Winnebago area of Wisconsin for years. Efforts have been made in the past to control other species of midges in various parts of the world. None of these methods, however, appears to be adaptable for use on Lake Winnebago's 137,000 acres.

Mechanical control, such as large light traps with electric grids or the burning of eggs (Lindquist & Roth 1950), has proved unsuccessful. The use of a surface oil to trap emerging adults (Gerry 1954, Lewis 1957) also has not been able to reduce tendipedid populations appreciably. Many insecticides have been tested for use against the larvae of midges. Orthodichlorobenzene, trichlorobenzene (Fellton 1940, 1941) and pyrethrum (Gerry 1951) are far too expensive to be considered for use on a large lake. The chlorinated hydrocarbons have been used with considerable success, but their use occasionally results in fish mortality and residue problems. TDE has been most extensively used (Lindquist & Roth 1950, Lindquist *et al.* 1951, Gerry 1954) because it is less toxic to fish than most other chlorinated hydrocarbon insecticides. BHC, DDT, and EPN have also been used successfully (Lieux & Mulrennan 1956, Lewis 1957) to control tendipedid larvae.

Because the organic phosphate insecticides generally create less of a residue problem, those commercially available were screened to determine their relative toxicity to *T. plumosus* larvae and to fish. Granulated insecticides were considered the most desirable, because the toxic ingredient can be concentrated on the bottom mud where the larvae of *T. plumosus* develop.

MATERIALS AND METHODS.—All tests were carried out in slate bottomed aquaria 10×20 inches and 16 inches high. Batteries of five aquaria were placed in large water baths so that a relatively constant temperature of 20° C. could be maintained. Mud from the bottom of Lake Winnebago was strained through a 20-mesh sieve to form a 2½-inch layer on the bottom of each aquarium. Water was then circulated through the aquaria until a 12-inch layer of clear water remained above the mud surface.

To test the effect of each insecticide on the larvae, 50 fourth instar *T. plumosus* larvae were placed in each tank and allowed 1 hour to burrow into the mud. The test insecticide, containing 5% technical material incorporated into 30/60 mesh AA-RVM attapulgite granules, was then evenly distributed over the water surface in each tank and allowed to sink to the bottom. Each insecticide was tested at rates of 0.01, 0.1, 1, and 10 pounds per acre. Air was bubbled into each aquarium 3 inches above the mud throughout the test period. After 5 days the mud was strained through a 20-mesh screen and the number of living larvae recorded. Any larva capable of movement was considered alive.

Table 1.—Per cent mortality of *Tendipes plumosus* and fathead minnows 5 days after the introduction of granulated insecticides.

	T. plumosus					FATHEAD MINNOWS				
	Lb./Acre Technical					Lb./Acre Technical				
INSECTICIDE	10	1	0.1	0.01	None	100	10	1	0.1	None
DDVP	98	84	90	10	10	16	8	4	4	12
Diazinon	96	98	40	8	10	100	12	12	0	12
Dipterex	92	82	78	12	4	16	4	4	4	4
EPN	100	100	94	18	24	100	48	8	8	0
Guthion	94	90	88	18	14	100	76	16	4	0
Ronnel[a]	90	36	20	28	26	100	4	4	12	4
Malathion	98	96	84	34	34	32	4	4	0	4
Methyl parathion	100	96	14	20	14	100	12	8	4	20
Parathion	100	98	28	44	32	100	16	0	12	8
Phosdrin	92	92	82	16	12	100	0	0	0	4
Demeton	100	100	26	16	14	56	8	8	12	12
TEPP	90	60	32	40	18	100	8	0	0	16
Tetram[b]	100	94	32	4	4	100	100	80	16	8
Thimet	100	94	18	8	0	100	88	0	8	0
Trithion	96	36	28	20	12	84	12	16	4	8
VC-13[c]	78	4	12	10	4	100	0	0	4	4

[a] New coined name for Dow ET-57; also called Korlan or Trolene.
[b] *O,O*-diethyl *S*-(2-diethylamino) ethyl phosphorothiolate [hydrogen oxalate salt].
[c] *O*-2,4 dichlorophenyl *O,O*-diethyl phosphorothioate.

To test the effect of each insecticide against fish, the same general proeedure was used. Approximately 50 larvae were placed in each aquarium to allow for the effect of the fish feeding on larvae killed by insecticide. The insecticide was introduced at concentrations of 0.1, 1, 10, and 100 pounds per acre prior to introduction of fish. Fathead minnows, *Pimephales promelas* Rafinesque, were used as the test fish. Twenty-five of these minnows were placed in each aquarium and the number surviving after 5 days was tabulated.

Replicated tests were run with the three most promising insecticides to determine more exactly their relative toxicity to *T. plumosus* and the fathead minnow. These tests were conducted in the same manner as the screening test, except that the test period was 7 days and any moribund larvae were considered dead. Four replicates were used at each concentration in a completely random design.

DISCUSSION.—From the results of the screening tests (table 1) the organic phosphate insecticides in general

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[2] Member of a cooperative research team composed of Dr. R. J. Dicke, Project Director, University of Wisconsin, and Dr. Daniel Palm and Dr. Jacob Shapiro, Wisconsin State College, Oshkosh. Supported in part by Winnebago Lake Fly Research, Inc.

were quite nontoxic to fathead minnows. Many of these insecticides also showed a considerable degree of toxicity to *T. plumosus* larvae. Of the phosphate insecticides showing a high degree of toxicity to *T. plumosus* larvae, Dipterex, DDVP, and malathion were the least toxic to the minnows and were selected for further testing. The results of these tests (tables 2 and 3) show more exactly the degree of toxicity exhibited by these insecticides toward *T. plumosus* and the fathead minnow. Malathion and Dipterex appeared especially favorable for lake treatment because of their low toxicity to mammals. However, all tests were run in only 1 foot of water and extensive field testing will be necessary before any recommendations for control can be made.

The low toxicity of malathion, Dipterex, and DDVP to fish also indicates the possibility of their use for the control of mosquito larvae in the vicinity of lakes and streams where chlorinated hydrocarbons cannot be safely used. It must be remembered, however, that thus far only the fathead minnow has been shown to be unaffected by large dosages of these insecticides. Additional tests, in both the laboratory and the field, involving other species of fish are needed to definitely determine how they are affected by phosphate insecticides.

Table 2.—Means of arc sin $\sqrt{\%}$ *T. plumosus* larvae surviving 7 days after introduction of granulated insecticides.[a]

Insecticide	Lb. per Acre of Technical					Standard Deviation
	0.100	0.075	0.050	0.025	None	
DDVP	(1.5) 4.90	(1.0) 4.05	(11.5) 18.48	(74.5) 60.25	(87.0) 69.40	7.30
Dipterex	(1.5) 4.90	(0.5) 2.03	(2.5) 7.60	(33.5) 31.48	(88.0) 70.35	12.18
Malathion	(1.0) 4.05	(3.0) 9.80	(23.0) 26.15	(51.5) 46.43	(92.0) 74.20	13.41

[a] Figures in parentheses represent unadjusted means of per cent *T. plumosus* larvae surviving after 7 days. Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different. 99% level ———— 95% level - - - - - .

Table 3.—Means of arc sin $\sqrt{\%}$ fathead minnows surviving 7 days after introduction of granulated insecticides.[a]

Insecticide	Lb. per Acre Technical					L.S.D. 0.95	Standard Deviation
	100	50	20	10	None		
DDVP	(83) 65.80*	(94) 77.95	(97) 82.05	(98) 84.25	(94) 77.70	11.67	7.75
Dipterex	(97) 84.53	(96) 82.05	(93) 75.08	(97) 81.38	(95) 78.93	ns	7.58
Malathion	(27) 23.70*	(90) 72.28	(98) 84.25	(99) 87.13	(98) 85.90	21.08	13.99

[a] Figures in parentheses represent unadjusted means of per cent fathead minnows surviving after 7 days.
* Significantly different from the control at the 95% level.

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Chemical Resistance of the Brown Dog Tick[1]

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In 1956, Hansens reported failure to control the brown dog tick, *Rhipicephalus sanguineus* (Latr.), with chlordane in a kennel in northern New Jersey. In other experiments, lindane applied to dogs at 0.045% gave control. This study, in the Dallas, Texas area, was designed to determine the approximate resistance to lindane and chlordane of field strains of this tick. The test method used is also reported.

While evaluating different materials for controlling brown dog ticks, Hansens (1956) used the following laboratory procedure: Filter papers were dipped in insecticide emulsions, dried overnight and used to line large test tubes. Live ticks were placed in the tubes and counts of mortality were made daily. Ticks were considered alive if they could turn over and walk.

Price (1957) used a different procedure in testing the susceptibility of resistant brown dog ticks. In this method the ticks were individually immersed in water emulsions of the test insecticides. The percentage of mortality was checked 1, 2 and 3 days after treatment. The criterion of death was the lack of physical response to mechanical manipulation as observed with a dissecting microscope (Price 1957a). In this study, chlordane at concentrations up to 0.5% and lindane at concentrations up to 0.03% failed to give satisfactory kill of engorged female ticks.

[1] Accepted for publication October 27, 1958.

TEST METHOD.[2]—Large engorged female ticks were collected and brought into the laboratory. As soon as convenient, and not over 24 hours after collection, the ticks were placed in a flat plastic sandwich dish and allowed to become active. Fresh insecticide emulsions were prepared at the desired concentrations. Depending on the number of specimens collected, 5 to 10 active ticks were picked at random and placed in a pouch made from a square of cheese cloth and dipped into the shaken emulsion for 10 seconds. Immediately following the dipping the excess liquid was shaken from the cloth pouch for 2 or 3 seconds before the specimens were spilled into a clean sandwich dish. After 5 minutes an absorbent paper bottom, with 15-mm. inked circles stamped on it, was placed under the specimens. Controls were run originally in an emulsion without toxicant, but this was discontinued after no increase in mortality time was noted. The controls held thereafter were untreated. The criterion of death was the inability to move out of the 15-mm. circle in 2 hours. All the ticks were positioned, legs down in the center of the circles and left undisturbed for the 2-hour period. The time of positioning was recorded and is the time used to establish the mortality lines. The specimens remaining inside the circles were held through all subsequent readings to eliminate the possibility of recovery from an anesthetized condition. Any tick succeeding in getting any part of her body over the line was counted as alive. The number of specimens dead at each reading was converted to per cent and plotted on logarithmic probability paper. The ordinate axis is per cent mortality and the abscissa is time, allowing the reading of a "lethal time" or L. T. value for any per cent mortality. The temperature during these tests was maintained at $75^{\circ} \pm 5^{\circ}$ F.

DISCUSSION.—Lacking specimens from a known nonresistant colony, the test was designed to compare populations to determine the difference in susceptibility to uniform concentrations of the test toxicants. Figure 1 shows the natural mortality of brown dog ticks under experimental conditions in the absence of toxicant. The results with lindane, figure 2, indicate a broad degree of susceptibility with some populations being susceptible and other populations being nonsusceptible. The results of the dipping with chlordane, figure 3, indicate a rather uniform, extremely low level of effectiveness. The exact degree of resistance cannot be determined by this method but the broad difference between populations indicates that, to a fair degree, chemical resistance exists in most of the populations of brown dog ticks in the Dallas area. A similar method was used by Laake & Williamson (1955) to assess the resistance of German cockroaches using residual surfaces where known nonresistant and resistant populations were available for comparison.

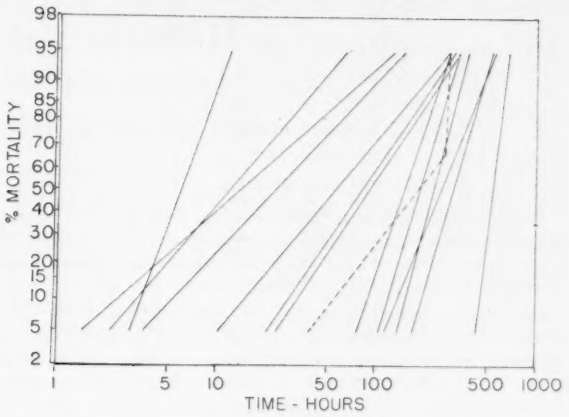

FIG. 2.—Mortality from lindane at 0.05% with broken line showing the earliest natural mortality.

The concentrations of the test insecticide emulsions selected were made for two reasons: the first was based on numerous reports from veterinarians and pest control operators that the concentrations had been effective in past years for the control of brown dog ticks; the second was that they could safely be applied to dogs as a dip.

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[2] Acknowledgment is due Dr. D. E. Howell, Oklahoma State University, for suggesting the criterion of death used in this procedure.

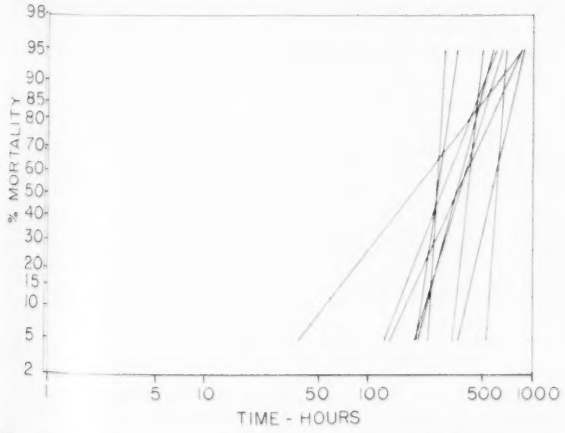

FIG. 1.—Natural mortality. The controls held during each run are plotted to indicate the time necessary for natural mortality.

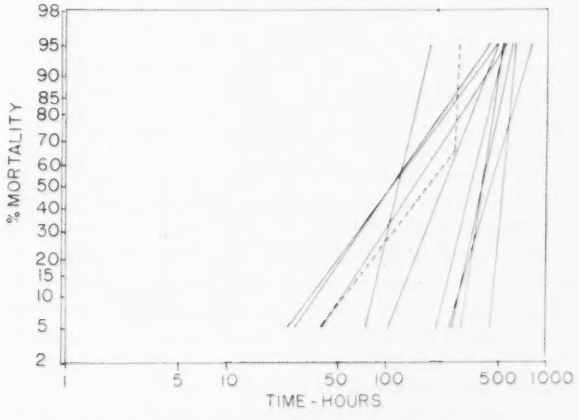

FIG. 3.—Mortality from chlordane 0.5% with broken line showing the earliest natural mortality.

Effects of Naphthenic and Paraffinic Petroleum Fractions of Comparable Molecular Weight on Transpiration of Eureka Lemon and Bearss Lime Plants[1]

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Abstract

Plants of Eureka lemon and Bearss lime were grown in pots in the glasshouse at a maximum temperature of 92° F. during the day and a minimum of 66° F. during the night. Transpiration was measured by determining the loss in total weight of the potted plants during a period of 7 hours from morning to afternoon; the surface area of the foliage was measured, and transpiration was expressed as milligrams of water transpired per square centimeter of leaf surface per hour. On the day following application of concentrations of 1.75% oil in aqueous mixture, transpiration of the oil-sprayed plants was reduced more than half that of untreated plants for both the naphthenic and the paraffinic oils and for both varieties of citrus. Oil-sprayed plants recovered with time. For narrow-cut fractions of comparable molecular weights of 306 and 308, recovery was faster in the plants sprayed with the naphthenic oil than in those with the paraffinic oil.

Intensive investigations by Chapman *et al.* (1943) and by Pearce *et al.* (1948, 1952) have demonstrated the relation of physical and chemical properties and of structural composition of petroleum oils to their efficiency against pests of deciduous fruit trees. Results of their investigations led to the recommendation of spray oils of highly paraffinic composition for use in New York State. Relations of increasing molecular weight and paraffinicity to the efficiency of saturated petroleum fractions for the control of scale insects and of mite pests of citrus have been reported by Riehl & LaDue (1952), Riehl & Carman (1953), and Riehl & Jeppson (1953). A greater likelihood of reduction of soluble solids of oranges with petroleum fractions of highly paraffinic composition and larger molecular size than in conventional California spray oils was reported by Riehl *et al.* (1954, 1957). Effects of a California spray oil on the transpiration of citrus plants have been reported by Riehl *et al.* (1958).

The experiments reported here were conducted for comparison of the effects of naphthenic and paraffinic composition of saturated, narrow-cut petroleum fractions of comparable molecular weight on the transpiration of citrus plants.

Materials and Methods.—A series of fractions of parent stocks of naphthenic and paraffinic composition were available in our laboratories. A fraction of each type was selected by matching the molecular weights as nearly as possible. Selected descriptive properties of the fractions are listed in table 1. The distillation ranges of the two fractions would occur within the third quartile of the distillation ranges of light-medium and medium-grade California spray oils. Since the fractions of naphthenic and paraffinic composition are narrow-cut and distill at temperatures in the upper part of the distillation range of light-medium and medium-grade California spray oils, the average molecular weight values of the fractions are slightly higher than those of medium-grade spray oils.

The plants used were obtained by propagating rooted stem cuttings of Eureka lemon and Bearss lime. The experimental procedures used in propagating and growing the plants, applying the oil spray mixtures, and measuring the transpiration are described by Riehl *et al.* (1958).

Table 1.—Selected properties of petroleum fractions.

Property	Petroleum Fraction	
	Naphthenic	Paraffinic
Molecular weight[a]	306.0	308.0
Paraffinic composition, %		
Lipkin Kurtz analysis	41.2	70.5
Waterman analysis	54.1	78.7
Distillation temperature, ° F.		
10%	638.0	655.0
50%	642.0	663.0
90%	647.0	668.0
API Gravity	29.4	37.4
Viscosity, S.S.U. at 100° F.	79.5	60.4
Unsulfonated residue	94.6	99.0

[a] Molecular weight determined by viscosity correlations of Mills *et al.* (1946)

Discussion of Results.—The means of the differences and the respective standard errors of the mean differences in milligrams of water transpired per square centimeter of leaf surface per hour between paired oil-sprayed and untreated check plants at various intervals after application are listed in table 2. These data show

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[2] The authors wish to express their appreciation to the Shell Development Company for the petroleum fractions used in these studies and for determinations of their physical and chemical properties; to J. P. LaDue and J. L. Rodriguez, Jr., of the University of California Citrus Experiment Station, Riverside, and to Mrs. Carol G. Wilson, formerly of this station, for valuable assistance in this work.

Table 2.—Inhibition of transpiration in Eureka lemon and Bearss lime plants after application of petroleum fractions of naphthenic and paraffinic composition at comparable molecular weights.[a]

Days After Treatment	Eureka Lemon				Bearss Lime			
	Naphthenic Oil		Paraffinic Oil		Naphthenic Oil		Paraffinic Oil	
	No. of Pairs	Mean Difference ± s.e. —d	No. of Pairs	Mean Difference ± s.e. —d	No. of Pairs	Mean Difference ± s.e. —d	No. of Pairs	Mean Difference ± s.e. —d
0[b]	10	0.9±0.7	10	−0.1±0.7	9	0.0±1.3	9	2.1±1.2
1	10	5.1±0.5	10	5.7±0.6	9	6.6±1.0	9	5.4±1.1
3	10	2.6±0.3	10	3.0±0.3	9	2.4±0.6	9	2.3±0.6
7	10	1.9±0.6	10	2.6±0.6	9	2.7±1.2	9	3.7±1.2
10	10	0.5±0.7	10	1.2±0.6	9	1.4±1.1	9	3.8±1.3
14	10	1.1±0.6	10	3.8±0.7	9	0.2±1.4	9	3.1±1.3
17	10	0.9±0.6	10	3.1±0.8	9	0.2±1.2	9	3.2±1.2
23	10	1.1±0.8	10	2.8±0.7	9	−0.7±1.7	9	2.8±1.3
30	10	0.3±0.8	10	1.9±0.6	9	0.5±1.3	9	2.7±1.2
37	10	0.1±0.5	10	1.2±0.5	9	−0.1±1.0	9	1.7±0.9

[a] Mean difference, in milligram of water transpired per square centimeter of leaf surface per hour, between paired oil-sprayed and untreated check plants, together with standard error of mean difference.

[b] Pretreatment.

effects similar to those found with the California spray oil (Riehl *et al.* 1958). A considerable reduction in transpiration occurred in leaves sprayed with either type of oil immediately after application, and this was followed by recovery in the rate of transpiration with time. The calculated data for correlations of transpiration vs. time for the two citrus varieties and types of petroleum composition are given in table 3.

The reduction in transpiration the day after spraying was approximately the same for both the type of oil composition and the citrus variety. However, the data of tables 2 and 3 show that recovery of transpiration is faster in plants sprayed with the naphthenic oil than in those with the paraffinic oil. The values of b for the slope of the regression line are larger for the naphthenic oil, and the differences in b for the two types of composition exceed the values of s_b in the trials with both varieties of citrus. The calculated values for differences in transpiration between oil-sprayed and untreated check plants, Y, 30 days after application indicate complete recovery in plants sprayed with the naphthenic oil, but not in those with the paraffinic oil. The differences in the values of Y 30 days after application for naphthenic vs. paraffinic composition exceed the values of the corresponding s_y.

On the other hand, if regression lines are plotted with data derived from the equations, no real differences are apparent in comparisons of the responses of Eureka lemon and of Bearss lime to the effects of either the naphthenic or the paraffinic oil on transpiration. This supports the conclusions of Riehl *et al.* (1958) that observed differences in recovery of transpiration associated with several citrus varieties following application of a California medium-grade spray oil were within the limits of the standard errors and were not more than might have occurred by chance within the conditions of the experiments.

Given properties of petroleum oils differ with changes in composition from naphthenic to highly paraffinic. Therefore, if a selection is made to obtain oils comparable for any given property, *e.g.*, molecular weight, differences in other properties must be accepted. The data of table 1 show that the paraffinic oil distilled at higher temperatures than the naphthenic oil. The slower rate of recovery of transpiration of the plants sprayed with the highly paraffinic oil may be associated with differences in the structural configuration of the hydrocarbon molecules or perhaps, in part, with differences in molecular size as indicated by distillation temperature. Experience in California shows that, in general, reduction or inhibition of the physiological functions of citrus trees following application of oil spray is greater with heavier grades of spray oils as indicated by higher distillation temperatures.

Initial inhibition and recovery of transpiration of Eureka lemon and Bearss lime plants sprayed with the naphthenic fraction agree well with the effects of a California medium-grade spray oil on the transpiration of these two varieties of citrus (Riehl *et al.* 1958). The paraffinic composition of the California spray oil is 47.0%; the average molecular weight is 295.

Table 3.—Calculated data for correlation of inhibition of transpiration vs. time.[a]

Citrus Variety and Oil	Regression Equation[b] ($Y=a+bX$)	s_b	r	F for Slope df=1,7	Calculated Value for 30 Days Y	Calculated Value for 30 Days s_y
Eureka lemon						
Naphthenic oil	$= 8.6-2.91121X$	0.26	−0.98	119.9**	0.31	0.18
Paraffinic oil	$= 7.5-1.97201X$	0.71	−0.72	7.8*	1.89	0.47
Bearss lime						
Naphthenic oil	$= 11.2-4.07843X$	0.55	−0.94	55.6**	−0.44	0.36
Paraffinic oil	$= 6.8-1.52335X$	0.57	−0.71	7.2*	2.48	0.38

[a] Data from table 2.

[b] Y = mean difference in milligrams of water transpired per square centimeter of leaf surface per hour, between paired oil-sprayed and untreated check plants; X = $\log_{10}$ of time in hours.

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Effect of Cultural Practices on Grasshopper Populations in Alfalfa and Cotton[1]

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Abstract

Surveys made in the Salt River Valley of Arizona in 1954–57 indicate that cultural practices, such as shortening the period alfalfa is grown on the same land and rotation with cotton or other cultivated crops, will reduce grasshopper populations in alfalfa. They also suggest that weed control would aid in controlling populations in alfalfa.

In the course of ecological studies on grasshoppers in central and southern Arizona during the period 1936–45, it was observed that populations increased with the age of the alfalfa stand. Plowing up the alfalfa and following it with a cultivated crop such as cotton usually reduced the population drastically. These trends were most noticeable in the Salt River Valley, where cotton and alfalfa are major crops and the migratory grasshopper (*Melanoplus bilituratus* (Walk.)) and the differential grasshopper (*M. differentialis* (Thos.)) are the dominant economic species. It was also noticed that the weediness of alfalfa fields often increased with the age and thinning of the stand.

Alfalfa fields are the leading habitat of economic grasshoppers in the warm valleys of southern Arizona, but an exclusive diet of alfalfa is inadequate for normal development of either the migratory grasshopper, as observed by Brett (1947), Pfadt (1949), and Barnes (1955), or the differential grasshopper, as found in rearing tests at Tempe. Other plants are needed to supplement alfalfa in the diet. Feeding tests at Tempe in 1953 and 1954 showed that diets consisting mostly of alfalfa but including one or more of the weeds commonly found growing in alfalfa fields were adequate for the migratory grasshopper.

Cultural Control.—Grasshopper control by cultural practices has often been mentioned in the literature. Shotwell (1930) found that heavy egg infestations of the migratory grasshopper were virtually destroyed by late-fall plowing and that populations were greatly reduced by summer fallow of infested land. Parker (1957) recommended cultural practices to destroy grasshopper eggs, restrain nymphs, and reduce damage. He also suggested grasshopper-immune crops and alteration of plant habitats as means of reducing infestations. Davis (1949) reported reduction of grasshopper damage by regrassing weedy field margins. On the other hand, Isely (1942) observed that infestations of the differential grasshopper increased in Arkansas areas where soybeans largely replaced cotton in the farming program.

Further investigation of the effects of cultural practices on grasshopper populations in the Salt River Valley of Arizona was started in the spring of 1954 and continued, as time permitted, through the summer of 1957. The studies were limited to fields of alfalfa and cotton, and the main objectives were to determine relative grasshopper populations in alfalfa and cotton, in alfalfa stands of different ages, in clean and weedy alfalfa, and to observe the effect of the age of alfalfa stands on the percentage of weedy fields.

Unfortunately for these studies grasshopper populations were unusually low in the Salt River Valley during this period. Already low in most fields in 1954 from application of insecticides to control grasshoppers or other insects during the preceding 10-year period, the numbers were reduced still further in the next 3 years by greatly increased use of malathion and other chemicals for control of the spotted alfalfa aphid. The summer grasshopper populations in alfalfa declined from 0.31 per square yard in 1954 to 0.068 per square yard in 1957, whereas during the period 1936–44 they averaged 0.6 to 2.65 per square yard. Notwithstanding the low populations, the type of crop, age of alfalfa stand, and whether fields were clean or weedy influenced grasshopper abundance.

Surveys.—Five surveys were made—a spring survey in alfalfa fields in 1954, summer surveys in alfalfa and cotton in 1954 and 1955, and summer surveys in alfalfa in 1956 and 1957. The alfalfa fields were grouped according to the age of the stand, as 1, 2, and 3 years or older. The grasshopper population in each field was estimated by visual inspection while walking across the field or counts by the pointer method (Barnes *et al.* 1953) on 20 one-square-yard areas usually about 10 to 15 steps apart and extending from near the margin into the central part of the field. The method of estimate and the spacing of samples depended on the height and density of the vegetation; the size, activity, and abundance of the grasshoppers; and the size of the fields. Alfalfa fields were designated as clean or weedy by visual inspection. Insofar as practicable, the fields of each crop or age group were representative of different local areas, soil types, and sizes.

Results.—The results of these surveys are summarized in tables 1–3. All averages in these tables are weighted averages.

The two surveys that included both alfalfa and cotton fields were made from August 23 to September 1, 1954, and from July 7 to August 10, 1955. As shown in table 1, the average population in alfalfa was about 15 times that in cotton. Evidently, a rotation from alfalfa to cotton, a crop that requires deep plowing in preparation for plant-

[1] Accepted for publication October 27, 1958.

[2] C. W. McLellan assisted in making grasshopper surveys and tabulating field data.

Table 1.—Comparison of grasshopper populations in alfalfa and cotton.

Year	Number of Fields	Number of Grasshoppers per Square Yard
	Alfalfa	
1954	48	0.310
1955	50	.235
Average	—	.272
	Cotton	
1954	24	0.016
1955	17	.020
Average	—	.018

Table 2.—Relation between the age of alfalfa stands and grasshopper populations.

Date of Survey	1 Year		2 Years		3–5+ Years	
	Number of		Number of		Number of	
	Fields	Grasshoppers per Square Yard	Fields	Grasshoppers per Square Yard	Fields	Grasshoppers per Square Yard
May 3 to June 14, 1954	13	0.077	9	0.611	14	1.528
Aug. 23 to Sept. 1, 1954[a]	23	.293	11	.550	14	.150
July 7 to Aug. 10, 1955	17	.116	19	.276	14	.326
July 3 to July 26, 1956	19	.084	17	.083	21	.177
July 8 to Aug. 13, 1957	9	.037	17	.049	28	.090
Total or average	81	.143	73	.261	91	.377

[a] In this survey one 1-year stand had four grasshoppers and one 2-year stand five per square yard, caused mainly by migration from an adjacent third-year stand that had recently been plowed. The respective populations in the remaining fields were 0.125 and 0.105 per square yard.

ing and clean cultivation during the growth period, is effective in reducing grasshopper populations.

Table 2 shows that the average population per square yard increased with the age of the stand. Table 3 gives grasshopper populations of alfalfa stands of different ages in both clean and weedy fields, based on data obtained in five surveys between May 1954 and August 1957. Regardless of the age of the stand, populations were decidedly higher in weedy fields than in clean fields. In all fields the average population increased as the age of the stand increased. The percentage of weedy fields also increased with the age of the stand.

In 1954, prior to heavy use of insecticides for control of the spotted alfalfa aphid, *Melanoplus bilituratus* and *M. differentialis* were clearly dominant in alfalfa fields in the Salt River Valley. In 1955 *Trimerotropis pallidipennis* (Burm.) was the leading species, followed by *Encoptolophus pallidus subgracilis* Caudell and *M. differentialis*. In 1956 the leading species were *M. bilituratus*, *T. pallidipennis*, and *E. p. subgracilis*, in the order given. The same species were dominant in 1957, but *pallidipennis* was more abundant than *bilituratus*. The decline in relative abundance of *bilituratus* and *differentialis* was probably caused by mortality from insecticides applied for aphid control in alfalfa fields and their margins. The favored habitats of *T. pallidipennis* and *E. p. subgracilis* are less restricted, and many nymphs or adults of these species could move into alfalfa fields from other crops or uncultivated land.

Table 3.—Relation between age of alfalfa stand and alfalfa populations in clean and weedy fields and percentage of weedy fields.

Age of Stand (Years)	Clean Fields		Weedy Fields		
	Number	Number of Grasshoppers per Square Yard	Number	Per Cent of Total	Number of Grasshoppers per Square Yard
1	50	0.069	31	38	0.261
2	38	.153	35	48	.377
3–5+	27	.207	64	70	.453
Average	—	.128	—	—	.387

These surveys indicate that grasshopper populations can be reduced, and the need for chemical control decreased, by cultural practices. Such practices would include shortening the period alfalfa is grown continuously on the same land and rotation of alfalfa with cotton or other cultivated crops. It is likely that weed control in alfalfa would aid in restraining grasshopper infestations.

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Control of the Corn Earworm in Grain Sorghum Heads[1]

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ABSTRACT

Single applications of several residual insecticides and Phosdrin were evaluated in South Carolina for control of the corn earworm, *Heliothis zea* (Boddie), in cases where it was infesting the heads of grain sorghum. Infestation data taken 3 and 14 days after treatment indicated that 1 or 2 pounds of DDT as spray or 2 pounds as dust and 2 pounds of Sevin as 10% dust were the only materials tested that gave good control throughout the crucial 2-week period. Present information indicates that these materials should be limited to use on sorghum grown for seed. Phosdrin at 0.5 pounds as a 2% dust resulted in fair control for 3 days, but failed for the longer period. Because of its short residual life, Phosdrin would be safe for use on feed sorghum. However, repeated applications would be needed to control the corn earworm.

One of the major insect pests of grain sorghum in South Carolina is the corn earworm, *Heliothis zea* (Boddie). Although this insect feeds down in the whorl of the leaves prior to the issuance of the sorghum head and causes the leaves to be ragged, the loss in yield of grain from this cause is comparatively minor. When feeding occurs within the sorghum head, kernels are partly or completely devoured, lateral branches of the panicles are severed, and the accumulated frass encourages the growth of mold within the head. In severely infested fields, loss of yield through missing or damaged kernels is often over 50%.

The period during which heads are damaged seldom extends over 2 weeks. Heads are rarely attacked until after they are about half through flowering, after which up to 20 early instar larvae may be found in a single head. As these larvae mature, the number is reduced through cannibalism or other causes until only 1 or 2 large worms are found in a head. After these larvae leave the heads to pupate in the soil, the heads are unlikely to become reinfested.

Burkhardt & Breithaupt (1955), DePew (1957), and Burkhardt (1957), working in Kansas, obtained 100% earworm control on grain sorghum after 3 to 7 days using Phosdrin at 0.5 pound, and 95 to 100% control using 2 to 2.75 pounds of DDT per acre. Other materials used were inferior. State and federal workers concerned with cotton insect research and control (1957) obtained good control of corn earworms on cotton using DDT, Sevin, and Thiodan.

Attempts to control this insect with DDT, on sorghum being grown for seed at the Pee Dee Experiment Station at Florence, S. C., in 1957, were quite successful. However, DDT residues render sorghum unfit for feed; therefore, a non-residual material is needed, since nearly all of the crop grown in the State is used as feed.

MATERIALS AND PROCEDURES.—When the plants in a field of Redbine 66 (combine type) grain sorghum started to flower, plots were laid off in uniform areas of the field. These plots consisted of single rows, 75 feet long, randomized and replicated eight times in test 1 and four times in test 2. Treatments consisted of single applications of insecticides applied to the heads after numerous early instar corn earworms were present. The heads were about half through flowering at this time. Sprays were applied with a compressed-air hand sprayer delivering 12 gallons per acre at 40 to 50 pounds pressure. Dusts were applied with a rotary hand duster delivering 20 to 25 pounds of dust per acre. Both sides of the rows were treated.

In test 1, DDT was applied as an emulsion at 1 and 2 pounds actual toxicant per acre, and as 10% dust at 20 pounds per acre. Sevin (N-methyl-1-naphthyl carbamate) was applied at the same rates. Thiodan was used as a spray at 0.75 pound per acre. Phosdrin was applied at 0.5 pound per acre as an emulsion and as a 2% dust at 25 pounds per acre. Two dust mixtures of malathion and Sevin were used; one contained 4%, the other 8%, of each material, at 25 pounds per acre. Three untreated plots were included in each replicate as checks.

Test 2 was in a portion of the field where the plants were delayed in flowering. Treatments were delayed for 10 days, after which only effective materials and rates, based on early data obtained in test 1, were applied. The materials were DDT, Sevin, and Phosdrin, and one check was included in each replicate.

Twenty-five heads were examined in each plot 3 days after treating. In test 1, these same heads were examined again 14 days after treating. Heads were selected on the basis of uniform maturity. Control was based on whether the heads were infested, rather than on the number of larvae found in the heads. One larva, regardless of instar, was sufficient to cause a head to be classed as infested.

Results.—Many heads of Redbine 66 sorghum are so tight that it seems impossible for a spray or dust to penetrate them. However, good corn earworm control was obtained inside these heads with some of the insecticides. The control might have been brought about because the larvae moved to the outside of the sorghum heads and thereby came in contact with the toxicant. The moving of the larvae to the outside of the sorghum heads could have resulted from their natural roaming or from being stimulated by the near presence of the insecticides.

The data as presented in table 1 show that in both tests 2 pounds of actual Sevin as a 10% dust gave 100% control after 3 days, and after 14 days was still giving 93% control. Sevin sprays at 1 and 2 pounds per acre resulted in 92 and 94% control, respectively, in test 1 after 3 days, but dropped to 62 and 74%, respectively, at the 14-day counts. At 2 pounds in test 2, Sevin controlled 91% of the worms, at the 3-day counts.

DDT dust at 2 pounds and DDT spray at 1 and 2 pounds gave as good control as Sevin spray in the 3-day counts, and held up better than Sevin in the 14-day counts, with 87, 91 and 93% control, respectively.

Thiodan spray used at 0.75 pound resulted in only 78% control in the 3-day counts, and 54% control in 14 days.

Phosdrin, which was a superior product in the Kansas tests, gave fair control for 3 days, but dropped to 55 and 60% for spray and dust, respectively, at 14 days.

The malathion-Sevin dust mixtures (4%+4% or

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Table 1.—Control of corn earworms in grain sorghum heads by single applications of insecticides applied as sprays and dusts. Florence, S. C., 1958.

Insecticide	Active Ingredient per Acre (Lb.)	Per Cent Infested Heads After Days Shown[a]			Per Cent Control After Days Shown		
		Test 1		Test 2	Test 1		Test 2
		3	14	3	3	14	3
DDT[b]	1	5.0	7.0	5.0	88	91	93
DDT[b]	2	0.5	6.0	4.0	99	93	94
DDT 10%	2	2.5	10.5	4.0	94	87	94
Sevin[b]	1	3.4	31.5	—	92	62	—
Sevin[b]	2	2.5	21.5	6.0	94	74	91
Sevin 10%	2	0.0	6.0	0.0	100	93	100
Thiodan[b]	0.75	9.3	38.0	—	78	54	—
Phosdrin[b]	0.5	7.6	37.0	12.0	82	55	83
Phosdrin 2%	0.5	9.0	32.5	8.0	79	60	89
Malathion 4%+Sevin 4%	1	3.0	22.0	—	93	73	—
Malathion 8%+Sevin 8%	2	0.5	32.5	—	99	60	—
Check	—	42.0	82.3	70.0	—	—	—

[a] Test 1 had eight replications; test 2 had four.
[b] Emulsion spray.

8%+8%) both gave good control of 93 and 99%, respectively, in the 3-day counts. At 14 days, however, the control was only fair, being 73 and 60%. This was in sharp contrast to the excellent residual control that resulted from Sevin dust alone.

Under the conditions of these tests, all of the insecticides tested gave at least 78% control at the 3-day counts, but DDT as spray or dust and Sevin as dust were the only materials that continued to give good control throughout the crucial 2-week period. A single application of either material gave dependable control of the corn earworm, and could be used on sorghum intended for seed.

Except for Thiodan, Phosdrin gave the poorest results, based on both the 3-day and the 14-day counts. However, for sorghum intended for use as feed, Phosdrin should be given consideration should the corn earworm become a problem. A single application would not be sufficient, but additional applications based on the infestation present might be practical on sorghum intended for use as feed. The present 3-day limitation between treatment and harvest indicates the lack of significant residues on treated crops after this period.

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BOOK REVIEW

The Insect Pests of Cotton in Tropical Africa, by E. O. Pearson assisted by R. C. M. Darling. Published jointly by The Empire Cotton Growing Corporation and The Commonwealth Institute of Entomology. 1958. Available from the latter; price 40S. Address: 56, Queen's Gate, London, S.W. 7.

We in this country might well be envious of the British in regard to the thoroughness with which they present available information. The Insect Pests of Cotton in Tropical Africa is a good example, being a thorough, factual account of the subject.

Preceding a discussion of the pests themselves, the authors trace the development of commercial cotton production in Africa through the wild or ancestral cottons to the present-day varieties and define the principal areas and ecological conditions governing where cotton is grown in that country.

A rather unique but highly interesting feature of the book is a section on origins of the pests of cotton. Although some speculation is injected into this discussion, it is an orderly and logical account of how cotton pests became associated with the plant. The discussion of the effects of insect attack on the yield of cotton is particularly good, as it brings into sharp focus the relationship between natural shed of the fruit of the plant due to environmental factors and damage caused by insects.

Keys are always helpful in providing an orderly means of associating plant disorders with their causative factors and the key which the authors have developed in this instance is particularly good since it describes such disorders by periods of plant growth. and parts of the plant, from seedlings to mature bolls.

The accounts of the principal pests are given by orders and the part of the plant attacked. The accounts of the various pests discussed are very thorough and the references cited to the literature are numerous. The eight colored plates are excellent and add a great deal to the value of the book.

As would be expected, the lepidoptera are the principal pests discussed, followed by the hemiptera. The discussion of the pests in these two orders occupies about 90% of the total pest account. Other orders discussed include the coleoptera, isoptera, thysanoptera, orthoptera, acarina, and myriapoda.

Of particular significance are the accounts of the various bollworms, in which the distribution and taxonomy, description of the stages, life history and feeding habits, host plants, natural enemies, status as pests, and general control methods are given for each of the principal species generally known. While some of these accounts are rather involved, a complete picture of their importance is given, including numerous references to the literature. No better general account of bollworms which attack cotton can be found, although of course the cotton bollworm or corn earworm of the United States, *Heliothis zea* (Boddie), is not discussed. The five colored plates showing the various stages of different bollworms and the damage which they cause are excellent.

The lack of agreement among taxonomists concerning nomenclature is forcibly brought to the attention of the American reader in the case of the pink bollworm, which is recorded under the genus *Platyedra*. It is of course known in this country as *Pectinophora* and our taxonomists are strong in their belief that *Pectinophora* constitutes a valid genus to which *gossypiella* should be assigned.

Perhaps the outstanding feature of the book is the extensive use of references. The authors evidently made an extensive review of the literature and in their discussions of the various subjects direct reference by number is made to corroborative or related publications. A total of 686 references are listed in the back of the book. Anyone interested in pursuing the subject further could use these to good advantages.

Without question this book is a valuable contribution to the subject of insects which attack cotton. Although it is confined to African species, many of these occur in other parts of the world. No economic entomologist can lose sight of the fact that an insect of economic importance to a given crop in one part of the world may also be a potential threat to that crop wherever it is grown.

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SCIENTIFIC NOTES

Emergence and Development of the Overwintered Generation of Peach Twig Borer Larvae[1]

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The life history and behavioral peculiarities of the peach twig borer, *Anarsia lineatella* Zell., are understood well enough to cover ordinary needs of investigations on control. But there are gaps in recorded information of its life history, the importance of which are not fully appreciated until costly outbreaks occur. One such gap is the lack of quantitative data concerning the movement of larvae into and out of foliage during each of its three principal generations. Information of this kind is pertinent to frequent questions about possibilities for control with unavoidably delayed petalfall sprays or with supplemental cover sprays. It was possible, during 1958, to study the temporal changes in the population of feeding larvae of one of these broods, the overwintered generation.

Almond orchards situated in parts of the Sacramento and San Joaquin Valleys of California tend to support dense infestations of twig borers, often without serious consequences to the crop. Larvae are easier to find in almond twigs than in twigs of peach, apricot or prune; and since untreated infestations sometimes are allowed to develop in almond orchards, certain of these are unusually favorable for studies of dense, undisturbed populations.

Emergence from Hibernacula and Later Development.—The peach twig borer winters on host trees as inactive, immature larvae, predominantly second instar forms burrowed beneath the soft bark of young wood, in shallow pockets or hibernacula (Bailey 1948). These have tell-tale "chimneys" so that sizeable samples of these are not difficult to cut from trees less than 5 years of age. Composite samples of approximately 250 hibernacula were taken from four varieties of almond trees at frequent intervals, beginning on January 29, 1958. These were dissected under magnification to determine relative numbers occupied and empty; those containing parasitized or otherwise dead larvae were excluded from the tallies. Sampling was continued until all hibernacula were empty.

The trend of emergence of overwintered brood, expressed as per cent of hibernacula empty, is shown by figure 1 (curve A). Prior experience has established that late winter samplings invariably contain some empty hibernacula—in this case 16% on January 29. This figure for the initial sample of the series approximates the static or midwinter condition and does not indicate that this fraction of the wintered brood was already active in the trees. The emergence of larvae proceeded most rapidly during the blooming period of almond or late pinkbud of peach. Only 5% of the hibernacula still contained larvae on March 24. A few larvae continued to emerge in diminishing numbers until April 18. The total period of emergence was approximately 11 weeks—considerably longer than was anticipated.

The maturing of the larvae and consequent decline in numbers of those in the feeding phase was traced by collecting samples of larvae from shoot tips and classifying them as immature or mature (full-fed). These determinations were plotted as percentages of mature larvae in the samples of those removed from twigs at successive intervals until no more larvae were found (fig. 1, curve B). The principal limitation of this procedure was that the twig-feeding population and size of samples declined rapidly near the end of the cycle. Initial samples fluctuated around 150 larvae, whereas totals for the two final samplings were 9 and 2 larvae, all mature.

Concluding Remarks.—The emergence and maturity trends described for the almond host are revealing in several respects. The beginning of the feeding phase for larvae which emerged late from hibernacula overlapped the maturity date for those which emerged early. The space of emergence was 80 days as compared with only 31 days required for the population to leave the foliage to pupate. Almost the whole of the population was concentrated in the twig-feeding phase during a 25-day period, March 17 (90% emerged) to April 11 (2% mature). The maturing of larvae in twig mines increased sharply after mid-April.

This information was obtained for almonds, the most favorable experimental host. Its applicability to peaches, on which crop losses are frequently critical, is affirmed by other data.

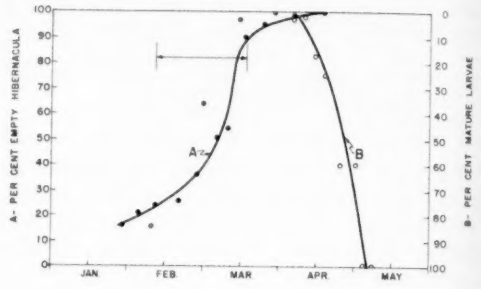

Fig. 1.—Buildup and maturity of populations of twig-feeding larvae of the overwintered generation of the peach twig borer, 1958. Curve A indicates per cent of hibernacula empty on dates sampled. Black dots—samples from almond trees, Arbuckle, California. Circled dots—samples from peach trees, Merced. Horizontal line, upper left, average duration of blooming period at Davis for major almond varieties (Wood 1947). Curve B represents per cent of full-fed larvae in samples of larvae recovered from almond twigs, Arbuckle.

That the trend of emergence of larvae from hibernacula on peaches closely follows the trend observed for almonds is indicated by four samples cut from peaches during this period of observation (fig. 1). Development of larvae of this generation on both of these hosts can also be related in terms of the earliest hatching date of eggs which produce larvae of the next generation. The dates on which youngest larvae of the May generation first appear in twigs has been determined for a number of years. Annual determinations made here since 1947 show that the dates of initial appearance of May larvae average approximately the same for the two hosts—May 14 for almond (9 years), May 15 for peach (6 years). Extremes for peach are May 12 to May 20; for almond these are May 10 to May 19.

Unusual amounts of rainfall and overcast throughout California during February to May, 1958, caused a regional difference in the timing of the May generation for 1958—May 13 for peach in the San Joaquin Valley and May 19 for almond in the more northern Sacramento Valley. The limited number of samples of hibernacula from peaches at Merced, San Joaquin Valley, indicated that the evacuation of hibernacula proceeded normally in this location during 1958, with a final result that the May generation arrived close to the average date in the San Joaquin Valley but 5 days later than average in the Sacramento Valley.

Recommended control practice involves spraying for twig borers during the pinkbud or 90% petalfall period. As a rule, almonds in the areas described are given petalfall applications about March 10 to 20, and peaches about March 20 to 30. During 1958, maximum populations of larvae continued to work in the foliage of these hosts until mid-April. Allowing for annual variations, it can be predicted that almost the entire population

[1] Accepted for publication November 10, 1958.

will be exposed to appropriate pesticides if applied not later than April 10. The likelihood of practical benefit from late applications appears to drop rapidly after mid-April.

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Malathion Residues in the Milk of Dairy Cows Treated for Horn Fly Control[1]

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Recent recommendations for the control of the horn fly, *Siphona irritans* (L.), on dairy cows (Anon. 1958) include only methoxychlor applied as 1 tablespoon of 50% dust per cow, and sprays of synergized pyrethrins and Lethane or Thanite. Availability to the dairyman of additional insecticides capable of producing satisfactory levels of horn fly control and at the same time involving no hazard of residues in the milk is desirable.

The report of Wells *et al.* (1958) on the effects of 0.5% and 1.0% malathion sprays and a 4% malathion dust formulation applied to dairy cows indicates that daily use of this pesticide results in only traces of malathion residues in the milk. The purpose of the work reported herein was to determine what insecticide residues in milk might be expected from malathion dusts and sprays applied to dairy cows at intervals necessary for satisfactory horn fly control.

PROCEDURE.—Two Jersey herds, milking from 21 to 24 cows, were used in these trials. One of the herds received three successive treatments with 4% malathion dust followed by an application of a 0.5% wettable powder spray. The other herd received a single treatment with 10% malathion dust.

Dusts were applied to the backs and flanks of the cows by hand at the rate of 2 ounces per cow. This resulted in an actual dosage of about 2.27 grams per cow in the 4% treatment and about 5.67 grams of malathion in the 10% treatment. The 0.5% spray was applied at the rate of 1 gallon per cow by means of a power sprayer operating at approximately 400 p.s.i. The actual dosage of malathion in this treatment is unknown. The cows were thoroughly soaked and considerable run-off of material took place.

Frequency of application of the 4% dust was determined by the rate at which the horn flies returned to the treated cows. Practical control was considered at an end when a herd averaged 30 flies per side per cow.

Additional treatments using 10% dust and 0.5% sprays were not made since malathion was detected in the milk after the first treatment.

All dust applications were made at the morning milking period, while the spray was applied about 4 hours prior to the evening milking.

The intervals at which milk samples were collected, in relation to time of treatment, are listed in table 1. Each sample consisted of a 2-quart pooled collection of milk from 12 to 14 cows. One quart of each sample was used in the analysis and the other was frozen and held for reference.

The milk samples were prepared for analysis by freeze-drying and analyzed in the manner described by Norris *et al.* (1958), which was found to have a sensitivity of 0.01 p.p.m. malathion in fresh whole milk. Recovery tests, in which malathion-free milk was fortified with malathion at levels ranging from 0.025 to 0.2 p.p.m., gave an average recovery of 82% based on 10 analyses.

RESULTS AND DISCUSSION.—The results of the analyses are summarized in table 1 where duplicate analyses have been averaged. Only two of the ten samples analyzed during the experiments with the 4% dust contained a detectable amount of malathion. When the control program was changed from a 4% dust to a 0.5% spray, definite milk contamination was detected. The resulting malathion concentration, 0.5 p.p.m., is in agreement with that found by Wells and co-workers (1958).

Table 1.—Insecticide residues in the milk and duration of horn fly control resulting from applications of malathion to dairy cattle.

HERD NO.	DATE	DURATION OF HORN FLY CONTROL (DAYS) 0–5 Flies per Cow	DURATION OF HORN FLY CONTROL (DAYS) Herd Average <30 Flies per Cow	SAMPLING TIME, HOURS BEFORE (+) AND AFTER (−) TREATMENT	MALATHION IN MILK (P.P.M.)
		4% Dust, Hand Application, 2 oz./cow			
1	7/2	5	14	+24	<0.01
				−12	<0.01
				−36	0.01
				−60	<0.01
	7/16	4	10	−12	0.01
				−36	<0.01
				−60	<0.01
	7/25	4	9	−12	<0.01
				−36	<0.01
				−60	<0.01
		0.5% WP, Power Sprayer Application, 1 gal./cow			
1	8/5	4	10	+ 8	<0.01
				− 4	0.50
		10% Dust, Hand Application, 2 oz./cow			
2	8/1	5	9	+24	<0.01
				−12	0.03
				−36	0.03
				−60	0.024

A 10% dust treatment also results in definite milk contamination (table 1). In this case the secretion of malathion appears to continue for at least 60 hours after treatment.

Horn fly control was essentially the same for the three formulations (table 1). These observations suggest that residues of malathion acutely toxic to horn flies were present on the cows for only 4 to 5 days. The balance of the interval of practical control is thought to be primarily a function of the potential of the fly population for recovery. During this period fly counts increased by increments of 4 to 5 flies per day with treatment again becoming necessary in 9 to 14 days.

Hand application of 4% malathion dust was found in these trials to be practicable. The duration of the effectiveness of the formulation, however, was not as great as that achieved by applications of 50% methoxychlor dust which Lindquist & Hoffman (1954) reported being effective for 3 weeks.

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[1] Oregon Experiment Station Technical Paper #1187. Accepted for publication November 15, 1958.

A Technique for Studying the Food Habits and Preferences of Grasshoppers[1]

GREGORY B. MULKERN and JOHN F. ANDERSON[2]

Previous investigations of the food preferences and feeding habits of grasshoppers have included a considerable amount of rearing of grasshoppers and plants under artificial conditions. This work has been valuable, but has many drawbacks associated with the artificial conditions. Anderson & Wright (1952) made an extensive study of the food habits of range grasshoppers by actually observing them feeding under natural conditions. However, this type of study is quite tedious and requires much time for each observation. Isely & Alexander (1949) reported on the possibility of analyzing insect food habits by examining the crops of the insects. They pointed out that food accumulates in the crop in a state of incomplete mastication and the fragments therein are often of such size and condition that they can be recognized.

In the study reported on herein a technique was developed by means of which the composition of the diet of grasshoppers can be analyzed by examination of the contents of their crops. The first step was to determine if different species of plants exhibited characteristics which permitted their identification in a masticated condition. Grasshoppers were confined on various plants for 2 days and then killed. Crops were removed and their contents examined. The results from these first experiments indicated that plants could be recognized in the crops and a broader program was initiated in which grasshoppers were individually caged on a larger number of species of plants in the field. Permanent slides were made of the contents of the crops of these grasshoppers and were used as "type" reference slides. Another method of obtaining type reference slides was developed using a Waring Blendor with a semi-micro container assembly. Plant samples were collected and preserved in 70% alcohol and then chopped in the blender to a particle size approximating that found in the grasshopper crop. Slides made from this material proved to be equal or superior to those of the contents of the grasshopper crop for reference purposes.

Many plants have various hairs, spines, serrations, etc., which can be used to identify them. In many cases plants are readily identifiable, while in some cases a plant can be determined as being one of a certain group of similar appearing plants.

For analysis studies, grasshoppers were collected with a sweeping net in the field or area selected and preserved in 70% alcohol. Then a list of the plants growing in the area and their approximate abundance was recorded. Plant samples were collected for reference material. Climatological and other pertinent data, such as condition of plants or field, were recorded on forms especially prepared for this purpose.

When the grasshoppers were to be analyzed, the list of plants for that field in which the grasshoppers had been collected was consulted and the type reference slides for those plants laid out. Usually, a cursory inspection of the slides was made to refresh the viewer's concept of the plant characteristics. Continual reference was made to these type slides during the study of the contents of the crops. The crops were removed from the grasshoppers and placed on microscope slides where the contents were removed and the crops discarded. The material was stained with double analine stain and a glycerin mount was made. The species and instars of the grasshoppers were recorded and the slides examined under a compound binocular microscope fitted with a mechanical stage. The contents were recorded on a form provided for that purpose. Each sample from an area consisted of approximately 100 grasshoppers.

The data obtained by this method are being utilized in a study of the food habits and preferences of grasshoppers as part of a project on the biology and ecology of North Dakota grasshoppers, results of which will be reported later.

It is believed that the method described will allow a comparatively rapid and accurate analysis of the food habits of grasshoppers. Neither the grasshoppers nor the plants are subjected to artificial conditions. Collections were made with a sweeping net and it is felt that a representative sample was obtained. By knowing the plants growing in an area, the plants that are not being eaten as well as those being eaten may be determined. In some cases where a plant in the crop cannot be recognized, it can often be identified by the process of elimination. The method allows for the collection and storage of grasshoppers during times favorable for this task. Analyses may be made later when convenient. Grasshoppers have been stored up to a year with no impairment of their value for analysis.

This method allows for much experimentation. By selecting fields in which the relative abundance of different plants varies, a comparison of the preferences of the grasshoppers for the various plants may be obtained. If all plants are eaten at random, they should appear in the crops in proportion to their abundance. If the grasshoppers are selective, the plants in the crops should reflect this by their disproportionate abundance.

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Control of Two-Spotted Spider Mites with Chlorbenside, Ovex, and Tedion[1]

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Chlorbenside, ovex, and Tedion are effective against the immature stages of spider mites. Ovex has been found to reduce spider mite populations on outdoor roses when used in combination with a fungicide and insecticide (Taylor *et al.* 1955). Chlorbenside has given the best results when applied early in the season to prevent buildup of mite infestations rather than to eradicate an active infestation (Cranham *et al.* 1953). Tedion was shown to have prolonged ovicidal action against mites in the laboratory (Huisman *et al.* 1955, Armstrong *et al.* 1955, Meltzer & Dietvorst 1957) and more recently proved highly effective on roses in the field (Ferguson 1958).

In the summer of 1957 experiments were conducted at Beltsville, Maryland, to compare the effectiveness of these materials for the control of the two-spotted spider mite (*Tetranychus telarius* (L.)) on outdoor roses and lima beans. All the experiments were set up in a randomized block design, with four replicates.

ROSE EXPERIMENTS.—Two experiments were conducted on roses, the first from May 15 to June 20 and the second from August 13 to September 17. Spider mites were transferred to the plants by stapling equal numbers of infested leaves to each plant and allowed to become established for 2 weeks before treatments were applied. Five weekly applications were made in each experiment.

Each plot consisted of six rose plants, three each of the varieties Helen Traubel and Red Radiance, 2½ feet apart each way in the plot with 5 feet between plots. The plants were mulched with a 1- to 2-inch layer of sawdust. Fertilizer applications were

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made during the season, and the plants maintained in good growing condition by periodic irrigations.

The acaricides in wettable powder sprays containing one fourth pound of active ingredient per 100 gallons of water were applied weekly with a 2-gallon compressed-air sprayer. All sprays also contained zineb (2 lbs. of 65% WP) for blackspot protection, DDT (2 lbs. of 50% WP) and lindane (1 lb. of 25% WP) for insect control, and Santomerse S (6.4 fl. oz.) as a wetting agent. Mite populations were compared by counting all living forms on five leaf disks nine sixteenths inch in diameter taken at random from each plot. Ratings from 1 to 10 on the basis of plant injury due to mite feeding were also made.

The results of both experiments are summarized in table 1. In the first experiment four weekly counts were made 1 week after the second to fifth applications. After the second count, the populations on the untreated check declined because of excessive injury, and the differences were not significant even though large reductions were evident on all the treated plots. The seasonal counts show Tedion and ovex to be more effective than Chlorbenside. Plants in all treated plots showed significantly less feeding injury than untreated checks. Tedion showed less injury than ovex, and both showed less than Chlorbenside.

In the second experiment three counts, made 1 week after the second, third, and fifth applications, show that all the treatments reduced mite populations below the untreated check. Tedion and ovex again appeared to be somewhat more effective than Chlorbenside. No injury ratings were made in this experiment.

BEAN EXPERIMENT.—In this experiment each plot consisted of a single 6-foot row of Fordhook 242 bush lima beans, with 5 feet between plots in the same row. The rows were 5 feet apart and the plots staggered so that the plots on one row fell opposite the space between plots of the adjacent rows. This arrangement of plots with clean-cultivated intervening areas prevented movement of mites, which takes place under our hot dry summer conditions when plots are arranged in solid plantings. The plots were infested with spider mites as for the rose experiments.

The three acaricides with Santomerse-S spreader were applied in wettable-powder sprays at the same rate as in the rose experiments but without a fungicide or DDT and lindane. Ovex was also applied as an emulsion. The first applications were made on August 13 and the last on September 17. Mite populations were compared by the disk method as for the rose experiment.

Table 1 shows a reduction in mite populations after the second application but no significant differences until after the third application. The seasonal counts were the same as after the last sampling. Plots treated with Tedion had significantly less feeding injury than those treated with ovex or Chlorbenside, but all treatments reduced injury below that on untreated plots.

Table 1.—Control of spider mites on roses and lima beans with weekly applications of Tedion, ovex, and Chlorbenside.

TREATMENT	MEAN NUMBER OF MITES PER PLOT AFTER INDICATED NUMBER OF APPLICATIONS					FEEDING INJURY INDEX
	2	3	4	5	Total	
First Rose Experiment						
Untreated	363	676	228	336	1603	9.5
Tedion	177	171	86	114	549	1.5
Ovex	150	182	62	179	573	3.8
Chlorbenside	276	254	198	272	999	7.3
L.S.D. (19:1)	154	244	n.s.	n.s.	424	1.8
Second Rose Experiment						
Untreated	72	—	250	68	392	—
Tedion	21	—	9	8	38	—
Ovex	35	—	23	17	75	—
Chlorbenside	99	—	49	30	178	—
L.S.D. (19:1)	n.s.	—	103	33	194	—
Lima Bean Experiment						
Untreated	124	190	—	213	527	10.0
Tedion	114	25	—	31	170	2.5
Ovex						
Suspension	116	50	—	79	245	5.6
Emulsion	105	33	—	119	256	5.0
Chlorbenside	98	53	—	64	214	5.3
L.S.D. (19:1)	n.s.	84	—	98	211	2.0

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A Method for More Accurate Determination of Air Volume Displacement of Light Traps[1]

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For over 100 years entomologists have been interested in the use of light traps for collecting insects. During this period considerable effort has been devoted to trap design and to contriving various sources of attraction. With suction traps, however, little attention has been given to standardization of mechanical performance. The earliest attempt to measure air flow was by Headlee and his associates following their initial use in 1932 of New Jersey light traps which had been designed for sampling adult mosquito populations (T. D. Mulhern, personal communication). The equipment consisted of a wheel anemometer and a stop watch. Mulhern (1948) adopted a 6-inch wheel anemometer mounted in a funnel which could be attached to the discharge end of the trap duct. In recent years, other workers have used more sensitive instruments such as vane and hot-wire anemometers, pitot static-tube and water manometers, and metrovic velometers (Taylor & Coleman 1955). Each instrument and method has its merits and limitations; however, regardless of the technique used, the measurement of air flow is complicated by the lack of uniformity in air velocity across the discharge duct.

After testing several experimental units an economical and easily constructed air tunnel was developed which can measure air velocity of suction traps with the desired degree of precision. The American Model trap (Mulhern 1953), a modification of the New Jersey trap, is used throughout California for standard adult mosquito population measurement. The equipment described here was developed for use with the American trap and

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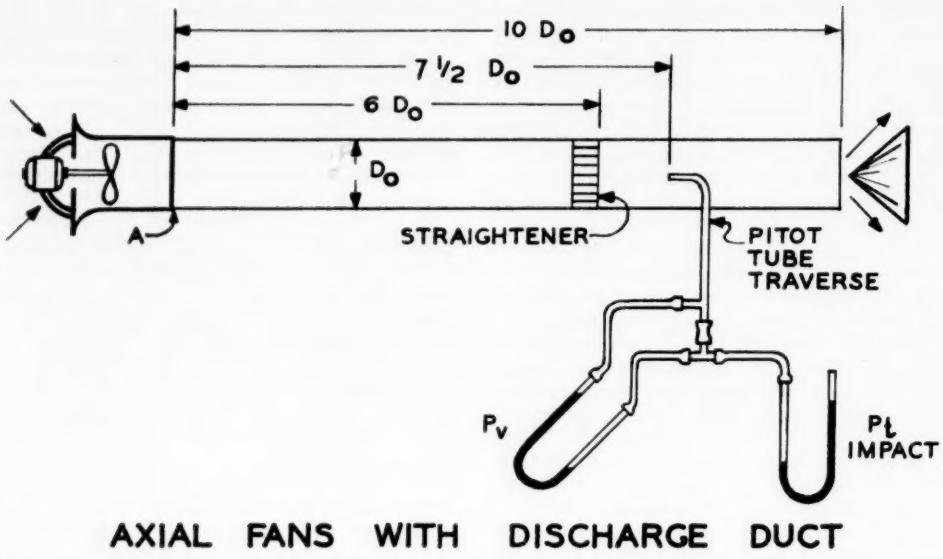

FIG. 1.—Specifications of air tunnel used for the measurement of air velocity and displacement in suction light traps.

follows the specifications outlined in the "Standard Test Code for Centrifugal and Axial Fans" as established by the Air Moving and Conditioning Association (1952).

AIR TUNNEL.—The dimensions of the air tunnel (fig. 1) are based on the diameter (D_0) of the discharge duct of the light trap. The American trap has a 9.75-inch duct diameter and the tunnel is, therefore, not less than 10 duct diameters in length (10 D_0). The tunnel may be made of galvanized sheet metal fitted on one end with a reducing collar for attachment to the end of the trap discharge duct (A). Necessary parts include an air flow straightening vane (straightener) and an air-measuring instrument (pitot tube traverse). The straightener is inserted in the tunnel at a distance of six duct diameters from the fan (6 D_0). The straightener consists of square cells, the widths of which may be from 7.5 to 15% of the duct diameter, and the length in direction of air flow is three times the width of a cell. In the model the straightener is placed 4 feet 9 inches from the fan. It is 4 inches long with cells 1.75 inch in width. Measurement of air flow is made at a point 7½ duct diameters from the trap fan (7½ D_0). The instrument most commonly used for measuring the air flow produced by axial fans is the double pitot tube and manometer. In the air velocity range of American Model traps the manometer readings are too low to permit accurate measurement. Therefore, a 4-inch wheel anemometer mounted on a permanent metal bracket within the air tunnel was used in place of the pitot tube.

The light trap and air tunnel are placed on wooden frames so that the entire assembly is in a straight line. All measurements are made in an enclosed room free from disturbing air currents. Three 5-minute readings are recorded for each trap and averaged to obtain a mean air flow in feet per minute. The mean air flow in linear feet per minute of 20 American traps tested was 287. The median flow was 300 with a range from 233 to 360. Air volume, expressed in cubic feet per minute, may be calculated by multiplying velocity in feet per minute by the duct area in square feet.

DISCUSSION.—The effect of different velocities of air flow on mosquito catches by light traps was tested in the laboratory using laboratory reared *Culex tarsalis* Coq. A room 6 feet 9 inches long by 4 feet 8 inches wide and 8 feet high was divided into two equal cubicles for light trap tests. In each test two traps differing in velocity of air flow were operated simultaneously for 12 hours. The results of five tests are presented in table 1. In all tests the trap operating at approximately 50% of the maximum air flow velocity collected far fewer specimens than the trap operating at maximum efficiency. There was no significant difference in the sex ratio collected by the two traps. Altering the position of the traps within the two cubicles by rotating the position of the traps during each test did not affect the catch.

Table 1.—Recovery of *Culex tarsalis* in light traps with controlled air flow.[a]

TEST NUMBER	TOTAL MOSQUITOES EXPOSED	COLLECTIONS IN LIGHT TRAPS			
		Trap A (238 f. p. m.)		Trap B (113 f. p. m.)	
		Number	Per Cent	Number	Per Cent
11	380	152	40	40	10
12	340	193	57	74	22
13	476	261	55	162	34
14	398	237	60	120	30
15	336	222	66	80	34
Total	1,930	1,065	55	476	26

[a] Source: State of California, Department of Public Health, Vector Control Records.

Traps operating 50% or more below their maximum air flow capacity after several weeks of field operation have been encountered. Air flow is frequently reduced by insects and debris that adhere to the screen funnel within the discharge duct or by the accumulation of debris on top of the motor mounts or on the screen guard placed between the trap hood and inlet duct. When standardization is a consideration, the maintenance of clean traps becomes as important as the use of traps of approximately equal air velocity ratings. Other factors that may affect air flow through traps but over which the investigator has very little control are the variation in voltage due to circuit overloading, and the nightly variations in wind velocity.

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Acceptability and Toxicity of Poisoned Baits to House Flies Resistant to Organophosphorus Insecticides[1]

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The resistance of house flies (*Musca domestica* L.) to organophosphrous compounds has become more of a problem since it was first reported in Denmark by Keiding (1956). In Florida as elsewhere, these compounds in baits, larvicides, or residues have been the preferred means of house fly control, but in 1956 it was found that malathion baits were not as effective as before (Lindquist 1957). Resistance was believed to be mainly physiological in nature; however, Kilpatrick & Schoof (1958) indicated that resistance to malathion baits may well be behavioral as well as physiological. To investigate this phenomenon further a laboratory study was undertaken with radioactive baits.

Malathion-resistant house flies from the Grothe colony, described by LaBrecque & Wilson (1957), reared in the laboratory to the F_{20} generation under malathion pressure were used to determine whether any behavioral resistance was expressed as an avoidance reaction to malathion and Dipterex baits. Baits were prepared by adding acetone solutions of malathion or Dipterex to sugar, mixing thoroughly, and drying. The baits, which contained 1% of toxicant, and the plain sugar standards were mixed with P^{32}-phosphoric acid so that the specific activity was about 8 microcuries per gram. One gram of the labeled bait or sugar was placed in a small metal container and exposed in a cylindrical screen cage containing 20 male or female flies that had been starved for 16 hours. Each test was run in quadruplicate. The flies were allowed to feed *ad libitum*, and water-soaked pads were placed on top of the cages during the test period. At 24 hours the mortality was determined, the bait was removed, and the dead flies were withdrawn from the cage and placed in vials. The live flies remaining in the cage were then killed with chloroform, and both groups of flies were assayed for radioactivity with an end-window G-M tube and scaler. Any fly that had fallen into the bait container was excluded from the test because of accidental contamination with the radioactivity. The counting data, counts per minute per fly, were transposed into milligrams of bait consumed by the individual flies.

The malathion bait killed 40% of the Grothe females, 85% of the Grothe males, and 100% of the normal flies. All the flies of both colonies were killed by the Dipterex bait. No mortality was observed with the sugar standards.

The results are given in table 1. The amount of unpoisoned sugar taken by individuals of each colony ranged from 2.4 to 19.2 mg. Only a few of the surviving Grothe flies took as much as 2.4 to 4.8 mg. of malathion bait, indicating a general avoidance of malathion by this colony since sugar was taken in much greater quantities. The fact that dead flies of both colonies had consumed less poisoned bait than sugar is not indicative of avoidance, since they presumably died as soon as the lethal amount had been ingested, but does not exclude the possbility of an equal level of avoidance in these flies. Both physiological and behavioral resistances were involved in the survival of the Grothe females. Half of the surviving females took 0.6 to 4.8 mg. of poisoned bait amounts equal to or greater than those taken by Grothe and normal females that died, an indication of physiological resistance. However, half the surviving females showed extreme avoidance of the poisoned bait, taking only 0 to 0.3 mg., an indication of behavioral resistance. None of the Grothe males showed such extreme avoidance; some died after consuming small amounts, but all those that survived consumed 0.6 to 2.4 mg., an indication of moderate avoidance combined with physiological resistance.

The results of the test with Dipterex bait run on female flies only, showed no evidence of physiological or behavioral resistance. Females from the normal colony fed on the sugar more voraciously than those from the Grothe group.

Under field conditions flies usually have a choice of food and freedom of search, and are not always hungry when exposed to baits. Since these experiments were run in the laboratory, and the flies were starved for 16 hours prior to testing, the results are not completely comparable to those that might be obtained in the field, as the avoidance reaction was tested more rigorously.

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Table 1.—Number of flies of the malathion-resistant (Grothe) and normal colonies consuming indicated amounts of malathion- and Dipterex-poisoned baits in 24 hours.

Bait or Sugar Consumed (mg.)	Malathion Bait								Dipterex Bait (All Females)			
	Grothe				Normal							
	Females		Males		Females		Males		Grothe		Normal	
	Dead	Living	Dead	Living	Dead	Living	Dead	Living	Dead	Living	Dead	Living
Bait												
0.0–0.3	—	8	4	—	11	—	9	—	1	—	—	—
0.3–0.6	—	—	7	—	4	—	1	—	1	—	—	—
0.6–1.2	1	2	6	2	3	—	—	—	3	—	1	—
1.2–2.4	3	1	2	4	2	—	—	—	7	—	12	—
2.4–4.8	6	4	1	—	—	—	—	—	3	—	2	—
Average mg. per fly	2.7	1.2	0.7	1.3	0.4		0.1		1.6		1.9	
Sugar												
2.4– 4.8	—	1	—	—	—	3	—	—	—	1	—	—
4.8– 9.6	—	5	—	10	—	5	—	3	—	11	—	5
9.6–19.2	—	3	—	—	—	2	—	7	—	3	—	10
Average mg. per fly	—	7.1	—	7.1	—	6.8	—	9.9	—	7.7	—	10.4

The degree of avoidance demonstrated in these experiments would therefore be expected to be one of the factors contributing to the lack of control with baits in the field, though less important than physiological resistance.

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A Method for Collecting Diapausing Boll Weevils for Hibernation Studies[1]

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Previous studies of the survival of the boll weevil, *Anthonomus grandis* Boh. during the winter months generally have been conducted in a manner described by Bondy & Rainwater (1942). Field-collected weevils were removed from growing cotton plants and transferred immediately to wooden frame, screen covered cages. This procedure was repeated in several cages during the fall. Bondy & Rainwater (1942), Reinhard (1943), Fenton & Dunham (1929), and other workers observed a definite trend toward greater survival of those weevils caged late in the fall. Investigators generally enclosed only the very active weevils from the field collections for hibernation studies (Sanderson 1907, Gaines, 1935). Sanderson concluded that the boll weevils could not be forced into hibernation as the insect's hibernation habits were dependent upon temperature. This worker supplied the weevils with cotton squares in the hibernation cages; yet the winter survival remained low.

Brazzel & Newsom (1958) studied hibernating weevils collected in ground trash in the winter and found them to be in a state of reproductive diapause. The examination of field collections by these workers in the fall of weevils from cotton indicated the presence of two physiological groups of boll weevils; an active reproducing group, and a group similar to those collected in the ground trash in which the insect's reproductive organs were in a state of physiological dormancy. The work reported herein was designed to allow the fall field-collected weevils to segregate in laboratory cages into these two groups in order that only the insects physiologically prepared for hibernation would be admitted to the hibernation cages for survival studies. The study was conducted during the fall of 1957 and spring of 1958 at the Entomology Research Laboratory at College Station, Texas.

MATERIALS.—Twelve hibernation cages were used in the investigation. These were wooden frame structures 4′×4′×4′ and covered with 16-mesh screen wire. A small door allowed entrance into the cages. About 50 pounds of Spanish moss was suspended from the top of each cage. The cages used to confine the weevils in the laboratory were similar to the hibernation cages but measured about 2′×2′×2′. A small tray 6″×12″ was placed in the bottom of each cage to hold moist sand and paper towels.

PROCEDURE.—The hibernation cages were placed in a row on the soil floor during September 1957 with 3 feet separating each cage. Cages 1 to 10, inclusive, were used to confine weevils which were collected from the field and caged immediately on the dates indicated in table 1. About 500 weevils were placed in each of these cages. These insects received no food after collection from the field. The boll weevils confined in cages 11 and 12 were allowed to feed for 2 to 4 weeks before confinement in the hibernation cages. On November 4, field-collected weevils were obtained from cotton and held in small cages in the laboratory at room temperature. The insects were allowed to feed daily on cotton squares. A tray containing moist sand was placed on the floor of each of the cages with about 12 folded and crumpled paper towels placed on the surface of the sand. The sand was moistened daily so that a portion of the towels remained damp at all times. Only weevils that ceased feeding and moved into the moist paper towels were placed in the hibernation cages.

Table 1.—Spring emergence of hibernating boll weevils at College Station, Texas.

1957 Date of Release	Cage No.	No. Weevils Released	Per Cent Emerged in 1958			
			April	May	June	Total
Unfed weevils immediately released						
Sept. 8	1	500	0	0	0	0
Sept. 25	2	500	0.2	0	0	0.2
Oct. 2	3	500	0.6	0.2	0	0.8
Oct. 9	4	500	1.4	1.2	0	2.6
Oct. 9	5	500	3.0	1.0	0	4.0
Oct. 16	6	500	1.6	1.4	0	3.0
Oct. 16	7	500	0.4	0	0	0.4
Oct. 30	8	500	0.6	0.2	0	0.8
Oct. 30	9	500	0.4	0.4	0	0.8
Nov. 4	10	479	1.2	1.2	0	2.4
Fed weevils collected in towels (field-collected Nov. 4)						
Nov. 13 / Nov. 21	11	207	13.0	6.3	0	19.3
Dec. 3	12	166	13.8	0	0	13.8

Most of the weevils remained very active in the laboratory cages. The insects were observed to feed freely on the food provided and to move about on the sides of the cages. On November 13, the paper towels were examined and 128 boll weevils were removed from the material and released in cage 11. These weevils were motionless and had assumed a very characteristic position on the paper towels. The insects pressed their bodies firmly to the material and, when agitated or disturbed, were much less likely to crawl or fly away than the actively feeding weevils in the cages. On November 21, another group of 79 weevils was removed from the paper towels and transferred to cage 11. On December 3, 166 weevils were taken from the towels and released in cage 12.

Emergence of the weevils was recorded during April, May and June and the sex ratio determined.

RESULTS.—The data in table 1 indicate that the highest percentage of emergence of unfed field-collected weevils occurred in cage 5. Four per cent of the original 500 weevils placed in this cage on October 9 were recovered during April and May. Less than 1% emergence occurred in 6 of the 10 cages where unfed weevils had been confined. The November 4 collection of weevils was divided into two groups. The first group, consisting of 479 weevils, was unfed after collection and placed directly into hibernation cage No. 10. Only 2.4% of these insects were recovered the following spring. The second group of weevils from this collection was fed fresh squares daily in the laboratory. On the dates indicated in table 1 those weevils that had ceased feeding and had moved into the moist paper towels in the feeding cages were transferred to cages 11 and 12. In the spring 19.3% and 13.8% of these weevils emerged from cages 11 and 12, respectively. It seems probable that in the past where hibernation work has been conducted many of the insects placed in the over-wintering cages

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were not those individuals physiologically adapted for hibernation. Weevil samples collected in the fall could be divided into three categories (a) reproducing individuals (b) weevils in the intermediate stages of diapause with little fat accumulation and (c) diapausing individuals with higher levels of accumulated fat. The reproducing weevils would die as soon as the food supply was terminated. The intermediate individuals would live longer but would have little chance of survival without additional food. The weevils that survive the winter would be individuals from category "c" that had fed sufficiently to be in "firm" diapause. These weevils probably were collected shortly before their normal movement into hibernation quarters. The procedure followed in the work reported herein was designed to furnish additional food to allow fat accumulation and attainment of "firm" diapause before the weevils were caged. The weevils collected from the paper towels were strikingly different in behavior from the actively feeding weevils. The fact that these weevils were very inactive in comparison with reproducing insects may have caused past investigators to consider them unfit for hibernation studies.

No weevils emerged in any of the cages during June and the test was concluded at the end of June. The sex ratio of the emerging boll weevils was determined during April and May. During April 52.6% of those individuals emerging were males and 47.4% were females. In May 60% were males and 40% females.

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Imported Fire Ant Mortality Due to Cold[1]

H. B. GREEN, *Mississippi Agricultural Experiment Station, State College*

The imported fire ant, *Solenopsis saevissima* var. *richteri* Forel, has spread northward through much of the Southeastern states in recent years. There has been some interest in states farther north concerning the northern limits of survival of this highly adaptable species. There are a number of factors which contribute to its establishment and subsequent spread in any given locality, but the effect of extreme cold is not well known. It has survived zero or near zero degrees Fahrenheit in east-central Mississippi during several years. In the course of other investigations in this area early in 1958, some mortality due to cold was noted.

Since there was not a great reduction in colonies per acre on test plots from the spring of 1957 to 1958, it may be assumed that the mortality due to cold did not account for substantial reduction in the number of colonies, but strength of many individual colonies was greatly reduced. This seemed to reduce the number of sexual forms usually produced during the spring.

The colonies most seriously damaged by cold were those that had moved to new locations and built mounds after early frosts. These mounds were therefore not reinforced by grass stems. There had been some winter rains followed by mild weather when the ants could build their mounds up with soft, fresh moist dirt. This was followed by freezing weather which caused icing in the mound dirt. It has frequently been observed that these ants do not ball together for warmth, but may be found immobile due to cold in all parts of the mound that is being used. Therefore, when a cold but thawing rain followed the freeze, the soft mound galleries collapsed on the ants. The cold rain was followed immediately by a hard, prolonged freeze. When the weather moderated it was found that only the ants in the lower levels of the damaged mounds survived. Large masses of dead ants could soon be found in soil depressions nearby as the survivors reworked the mounds.

This combination of weather conditions which caused the great mortality described above does not occur often in Mississippi; but it might farther north.

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Rubber Latex Paint for Separating Pesticide Deposits on Upper and Lower Surfaces of Leaves[1]

G. F. MANSON and J. R. W. MILES,[2] *Entomology Laboratory, Chatham, Ontario, Canada*

A study of the distribution of pesticides applied to tobacco plants by aircraft and ground equipment necessitated separation of deposits on the upper and lower surfaces of leaf samples. Several methods of separation were tested and discarded in favor of painting upper surfaces of leaves with a rubber latex emulsion[3] before applying the pesticide.

METHODS.—At selected stations in the area to be treated, half of the upper surface of leaves was painted with latex. A stiff bristle paint brush was used. The rubber film cured in about 2 hours, adhering closely to the leaf surface. After treatment, leaf disks of known area were punched from the painted part of the leaf, peeled from the latex film with forceps, and placed directly in collecting jars. Similar samples were taken from the unpainted part of each leaf for comparison. The pesticide was extracted in the laboratory and analyzed by specific analytical methods. The deposits were expressed in micrograms per square centimeter of leaf surface.

DISCUSSION.—Dyed sprays used by Sanders (1953), Chamberlin (1955), Deonier (1955), and others to measure spray distribution, were collected by pans, plates, or cards, and were not applicable to this study. Earlier attempts at Chatham to use colored dusts were also unsuccessful as it was impossible to remove the deposit without extracting leaf pigments, which interfered with the direct colorimetric readings.

Dr. A. W. A. Brown, University of Western Ontario, London, who cooperated in the preliminary studies at Chatham, measured dust deposits from aircraft gravimetrically using paired oil-coated plates. These were set horizontally in sawcuts in wooden stakes distributed over the treatment area. Based on a composite sample from 24 sets of plates, the upper surface deposit was 78 milligrams, the lower surface deposit 26 milligrams, or a ratio of 3 to 1. In the same experiment when the deposits on tobacco leaves were measured chemically on 40 leaf tissue samples, the average upper leaf residue was 9.7 micrograms and the lower leaf residue 1.4 micrograms, or a ratio of 7 to 1.

This experiment and earlier, unpublished work by Mr. J. E. Armand of the Chatham staff using paired cards at different levels showed that deposits on artificial surfaces differ from those on foliage.

Paper masks attached by adhesive tape or staples to tobacco

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[2] Entomologist and Chemist.

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leaves were easily torn by wind, especially when wet with rain or dew. They sometimes distorted the leaves and seldom adhered close enough to the leaf surface to eliminate all possibility of contamination.

Attempts to sample deposits on the upper surfaces of leaves by painting latex on the lower surfaces of tobacco leaves were unsuccessful. The rough-veined lower surface prevented formation of a uniform film and interfered with peeling the leaf tissue from the rubber.

The latex method as recommended proved satisfactory in sampling tobacco for dust or spray deposits applied by aircraft or ground equipment. As rubber film is unaffected by weather, it may be applied well in advance of the pesticide treatment if required. With minor adaptations it should be possible to use this method on most crop foliage.

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Soil Drenches for Control of the Narcissus Bulb Fly[1]

CHARLES F. DOUCETTE, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

Preplanting treatments of narcissus bulbs, either by short soaks or with dust formulations of heptachlor, aldrin, chlordane, or dieldrin have been found to give good protection against larvae of the narcissus bulb fly, *Lampetia equestris* (F.) (Doucette 1953). Frequently, however, bulbs may be planted without such treatment, or they may be left in the ground for several years, as in garden plantings. Efforts have been directed towards finding means of preventing bulb fly injury to such bulbs. Field sprays with oil emulsions, alone and combined with other insecticides, have been found to give moderate protection (Schopp *et al.* 1943). In these tests the sprays were directed at the bases of the plants, the area of oviposition, the rate of application was approximately 1 gallon to 100 feet of row. Naphthalene flakes applied to the soil around the plants were also effective (Doucette 1948).

The present paper reports on tests at Sumner, Wash., with drenches, which give more penetration of the insecticides into the soil around the necks of the bulbs than do sprays. The drenches were applied with a 3-gallon compressed air sprayer without a nozzle, the stream being directed at the neck area of the bulbs. Walking along the row was timed to apply about 2 gallons to 30 feet of row.

Drenches were first tested on narcissus bulbs in 1955, and further experiments were made in 1957 and 1958. Each year three materials were used—aldrin and heptachlor emulsifiable concentrates and Dipterex as a 50% soluble powder. Aldrin was used at 2 pounds and heptachlor and Dipterex at 1 pound per 100 gallons. There was a single application in each season—on June 8, 1955, May 11, 1957, and May 16, 1958. These dates represented the beginning of the period of adult activity, as indicated by emergence from infested bulbs in field cages. In 1955 and 1957 each material was applied to four narcissus varieties, with adjacent untreated bulbs for each lot. The 1958 tests were made on replicated plots of one variety. At maturity the bulbs were dug and examined for infestation. The infestation data are summarized in table 1.

Table 1.—Narcissus bulb fly infestations in narcissus bulbs treated with soil drenches.[a]

INSECTICIDE	1955		1957		1958	
	Infested	Total	Infested	Total	Infested	Total
Untreated	48	730	94	373	71	100
Aldrin	0	220	0	151	1	100
Heptachlor	0	190	3	110	2	100
Dipterex	1	160	0	112	2	100

[a] Figures indicate number of bulbs.

These results indicate that drenches of aldrin, heptachlor, and Dipterex will give a high degree of protection against the narcissus bulb fly. The volume of water needed would make the method impractical for large-scale use, but it would be very desirable for treatment of high priced novelty varieties or hybridizing stocks, and especially suitable for gardens where the bulbs are left in the ground from year to year. A single application at the start of adult activity will give the desired protection. The appearance of adults will vary with the area and the season. In warm sections it can be expected to start about mid-April, and in most areas early in May unless the season is unusually late.

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[1] Accepted for publication December 19, 1958.

A Floating Pan Trap for Insects Associated with the Water Surface[1]

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The trap described herein was designed primarily for sampling the adult population of *Hydrellia griseola* Fallen in rice fields. This species of fly is most commonly observed on the water surface film actively moving about in search of food, oviposition sites, and mates. An open floating trap was selected after several unsuccessful trials with various funnel-type, bait traps.

The trap (fig. 1) consisted of an aluminum pan 8 inches in diameter and 1¼ inches deep, set in a circular hole cut in a 12-inch square of wood 1½ inches thick. Three screws through the rim of the pan fixed it to the wooden float. A depression 2½-inches in diameter and approximately three-eighths inch deep was formed in the bottom of the pan to centrally locate the trapped insects. Two ½-inch eyelet screws were placed at opposite corners of the float so that suitable lengths of one-quarter inch doweling could be vertically inserted through the eyelets and into the substrata below the water. This arrangement prevented horizontal drift of the trap but allowed vertical movement as the water level changed.

Water was placed in the pan until the level was approximately one-quarter inch from the top, and 1 to 2 milliliters of a wetting agent was added to the water. "Tergitol-7" was the wetting agent primarily used, although several others were tried and also found to be effective. The reduced surface tension of the water caused alighting insects to sink to the bottom of the pan.

Contents of the traps were emptied every 1 to 3 days. Large insects were removed with a forceps. The majority of the

[1] Accepted for publication December 22, 1958.

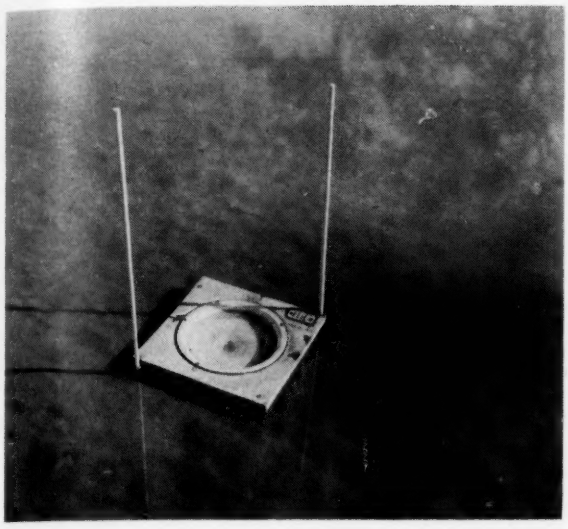

FIG. 1.—Floating pan trap.

trapped specimens were picked up by a glass suction tube 10 inches long by 1 inch in diameter, drawn to a one-quarter inch tip. The contents of the syringe were then passed into a second glass tube, 1 inch in diameter, that contained a 40-mesh, wire filter. This procedure was executed over the trap so that the water containing the wetting agent was returned to the pan. The filter tube was then inverted and the specimens washed with alcohol into a vial for storage.

The traps should be located at a site sheltered from heavy wave action. A position 1 to 2 feet from the shoreline usually discouraged small animals from submerging the trap. It was necessary to make collections at least every 3 days to prevent decomposition of the specimens and replace evaporated water.

This trap gave a very satisfactory index of the adult population of *H. griseola*. It was not selective for this insect, however, and considerable time was required for sorting. The traps nonselectivity was used to advantage in that closely related species and parasites were also recorded. Collections from this trap included representatives from the following orders: Collembola, Ephemeroptera, Orthoptera, Odonada, Homoptera, Hemiptera, Thysanoptera, Coleoptera, Diptera, Hymenoptera, and Lepidoptera. The wide representation of collected insects suggests the possible use of this type of trap for studies of other insects associated with the water surface. No special attractive feature was used in conjunction with this study, but modifications such as altering the color of the pan or the addition of baits to the water might provide selectivity. Screens of different mesh over the pan might also be used to advantage.

Lindane in Milk from Sprayed Cows[1]

GEORGE W. WARE and L. O. GILMORE[2]

Several states presently recommend or have recommended lindane for the control of sarcoptic mange on dairy and beef animals. The recommended rates of application varied from two sprays of 1½ pounds of 25% wettable powder in 100 gallons of water 2 weeks apart, to one spray of 3 pounds of 25% wettable powder in 100 gallons. The methods of application varied from low pressure spraying, with added household detergent to give thorough saturation, to 250 p.s.i. for driving the spray to the skin.

It was the purpose of the experiment results of which are reported here to determine if the presence of a household detergent in the spray would facilitate penetration of lindane through the skin and consequently result in more rapid appearance and perhaps prolonged occurrence in the milk. At the same time the problem of how long milk from sprayed animals should be withheld from consumer channels because of contamination following lindane spraying could be answered.

On February 24, 1958 four dairy animals at the Ohio Agricultural Experiment Station, Wooster, were sprayed with a concentration of 1½ pounds of 25% lindane wettable powder in 100 gallons of water. Two animals, nos. 1343 and 1326, weighing 931 and 700 pounds, respectively, were each sprayed with 1½ gallons of the mixture. Two animals, nos. 1342 and 1265, weighing 928 and 950 to 1000 pounds, respectively, were each sprayed with 2 gallons of the mixture which also contained a household detergent, Tide, at the rate of 2 pounds per 100 gallons. The gallonage variation was not intentional, but was due rather to body surface saturation requirements.

The animals were sprayed individually with a portable, wheel-mounted, 12½-gallon Bean sprayer, at 40 p.s.i. The animals were thoroughly wetted, except that the face and udder were avoided. Precautions were taken to prevent the feed trough from being contaminated.

Pint prescription bottles were used to take pint milk samples from the sprayed and one untreated check animal immediately following spraying and 1, 2, 3, 4, 5, 6, 11, 14, and 17 days after treatment.

The milk samples were preserved by the addition of 1 ml. of 40% formalin solution and held at 40° F. The samples were removed to the residue laboratory and lindane determinations were conducted by the Schechter-Hornstein method (1952), as modified by Wylegala *et al.* (1956) and by Ware & Gilmore (1958).

From figures 1 and 2, it will be seen that lindane appeared at its highest level in milk from all animals 1 day following spraying and diminished logarithmically with time. Lindane was detect-

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[2] Assistant Professor, Department of Entomology, and Professor, Department of Dairy Science, Ohio Agricultural Experiment Station, Wooster, Ohio, respectively.

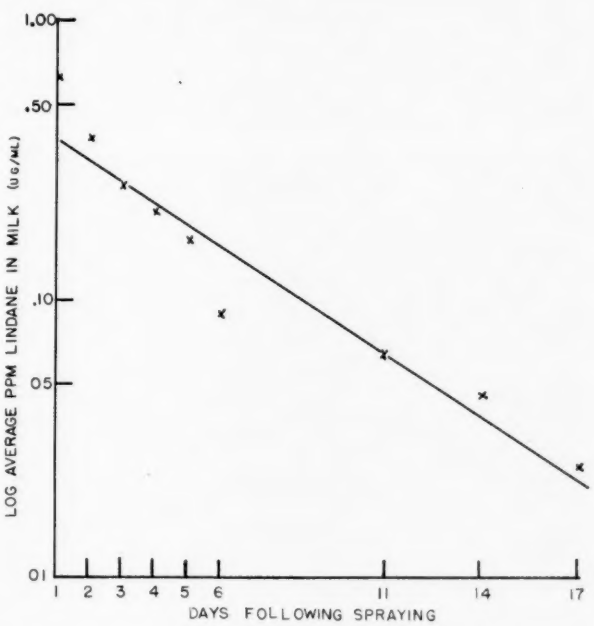

FIG. 1.—Average parts per million of lindane found in four test animals' milk after spraying with lindane.

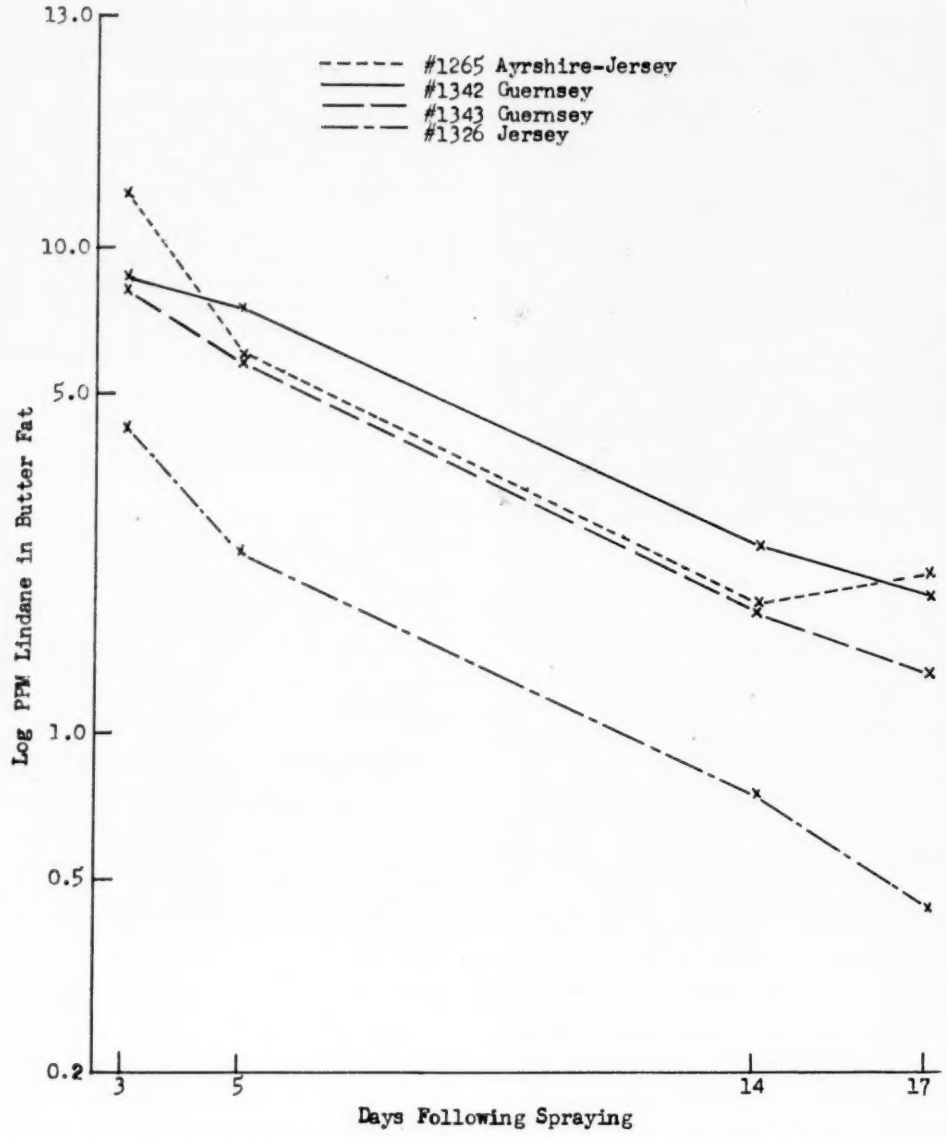

FIG. 2.—Parts per million of lindane in butter fat of four individual test cows after spraying with lindane.

able in the milk of all treated cows 17 days after spraying. From the small amount of data presented, the presence of a detergent in the spray did not appear to affect the appearance or disappearance of lindane in the milk.

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Granuloses in Two Alaskan Insects[1]

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In 1928, King and Atkinson reported on a supposed bacterial disease, and on a disease of unknown origin occurring in larvae of the red-backed cutworm, *Euxoa ochrogaster* (Guenée), in Saskatchewan. They assumed that the bacterial disease was caused by *Bacillus noctuarum* White because the symptoms they observed in *Euxoa* were similar to those described by White (1923) in other cutworms (*Feltia*). However, they did not identify or describe the supposed bacterium involved.

In June, 1958, we received from Richard H. Washburn of the Alaska Agricultural Experiment Station, a number of diseased specimens of *Euxoa ochrogaster* that he had collected during an

[1] Accepted for publication December 8, 1958.

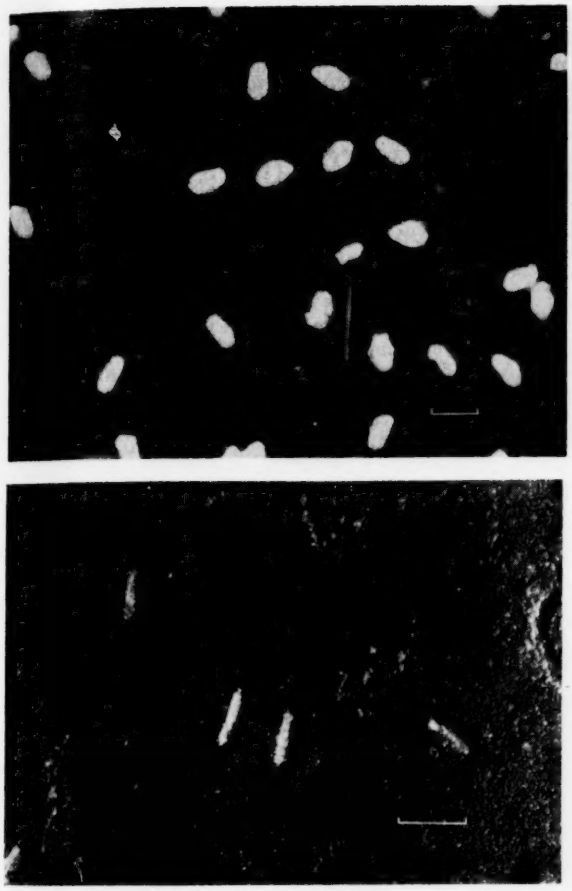

FIG. 1.—Electron micrographs of capsules (upper) and virus rods (lower) of granulosis in *Euxoa ochrogaster* (Guenée). Virus still retained in intimate membrane. Preparations shadowed with palladium. Scale shown equivalent to 0.5 micron.

outbreak of this insect in Matanuska Valley, near Palmer, Alaska. Upon examining the larvae we found them to be infected with a granulosis virus. It is possible that this virus was the true etiological agent of one of the diseases described by King and Atkinson.

The virus capsule, as well as the virus rod itself, is typical of those characterizing granuloses generally. The average size of the capsule was found to be approximately 250 by 440 millimicrons; while the virus rod itself was approximately 60 by 360 millimicrons. Electron micrographs, taken routinely in the course of making the original diagnosis, are shown in figure 1.

Diseased specimens of another Alaskan insect, the spear-marked black moth, *Eulype hastata* (Linnaeus), were sent to us in August, 1958, by G. L. Downing of the Alaska Forest Research Center. They had been collected southeast of Fairbanks. According to Mr. Downing, an outbreak of this insect has occurred over a gross area of 5,829,000 acres, causing moderate to heavy defoliation of white birch. A short time before the diseased specimens were collected, a sharp population decline was noted when the insects were in the third and fourth instars. Some of this decline is known to have been caused by insect parasites, but it is believed that disease was probably the primary cause of the decline. Dead and dying larvae were seen hanging from the foliage. The summer had been warmer than usual with an average humidity of about 65%.

Upon examining the body contents of the diseased larvae with an ordinary light microscope we observed the presence of large numbers of capsules typical of granulosis virus infections. Electron microscope observations confirmed the diagnosis of granulosis, revealing the presence of rodshaped virus particles. In one lot of 14 larvae, six were found infected with a combination of *Entomophthora* sp. and Microsporidia (*Nosema*), seven were infected with the granulosis virus as well as the Microsporidia, and one specimen appeared to have died of a combination virus-bacterium (gram-negative small rod) infection. However, the granulosis predominated in all of the several lots examined.

The approximate size of the capsules was found to be 200 by 360 millimicrons; while the average dimensions of the virus was about 60 by 320 millimicrons. Measurements were calculated from palladium-shadowed electron micrographs. (See fig. 2).

Unfortunately, few virus rods free of their intimate membranes were found in the electron micrographs, and the inadequacy of infectious material available precluded the making of additional electron microscope preparations at this time. Accordingly, the sizes of the virus rods described in this paper should be regarded as rough approximations only; in all likelihood, for instance, the naked rods may be slightly narrower than 60 millimicrons.

Both of the insects concerned in this report can be of considerable economic importance, hence the finding and identification of natural control factors, such as the viruses involved, is of sig-

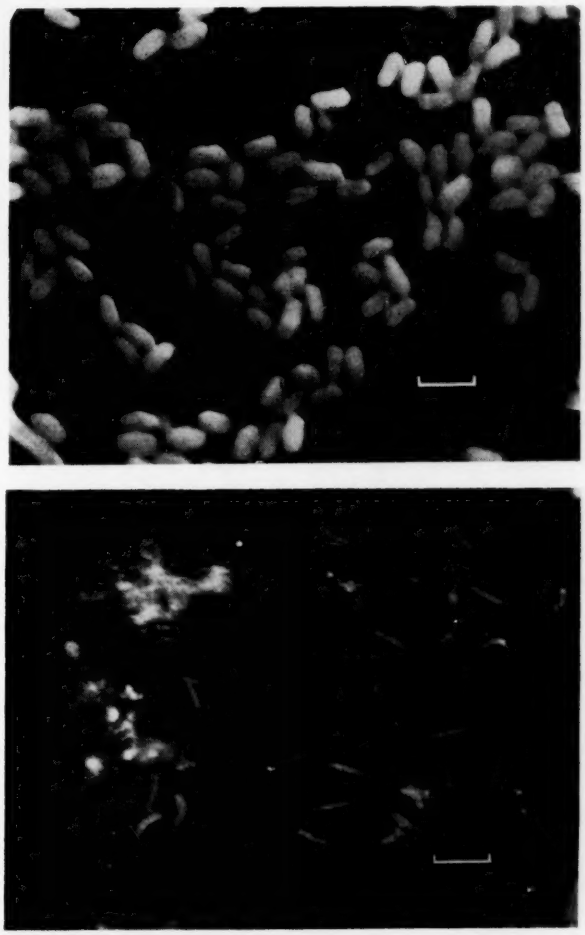

FIG. 2.—Electron micrographs of capsules (upper) and virus rods (lower) of granulosis of *Eulype hastata* (Linnaeus). Preparation shadowed with palladium. Scale shown equivalent ot 0.5 micron.

nificance. In the case of *Euxoa ochrogoster*, the virus herein reported may have been the cause of one of the diseases reported in this insect in 1928 by King and Atkinson. The granulosis of *Eulype hastata* has not heretofore been reported.

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Insecticide Resistance in the Salt-Marsh Sand Fly *Culicoides furens*[1]

CARROLL N. SMITH,[2] A. N. DAVIS,[2] DONALD E. WEIDHAAS,[2] and E. L. SEABROOK[3,4]

The salt-marsh sand fly *Culicoides furens* (Poey) breeds throughout the year in the moist soil of tidal marshes along the east coast of Florida. The adults are capable of migrating several miles and cause severe annoyance to inhabitants within this radius. In some areas the presence of sand flies has discouraged tourist trade and hindered economic development.

Early work on the control of sand flies consisted of ditching, diking, and pumping of tidal marshes (Hull & Dove 1935, Hull *et al.* 1939, 1942). These practices apparently caused a marked reduction of sand flies in some areas but in other areas they were of doubtful value.

Aerial or ground spraying or fogging with certain insecticides will curtail adult activity and provide temporary relief from annoyance. However, reinfestation usually occurs within a few hours to a few days, making it necessary to re-treat frequently. Such operations are expensive and generally impractical.

Because of the absence of suitable control measures, the Co-Operative Sand Fly Research Unit was established in 1949 to study the biology and ecology of salt-marsh sand flies with the ultimate aim of devising a method of control. This unit was under the technical supervision of the U. S. Dept. Agric. Orlando, Fla., laboratory and was supported by funds allotted by the Division of Entomology of the Florida State Board of Health and the Anti-Mosquito Districts of Dade, Broward, Palm Beach, and Indian River Counties. Tests conducted by and in conjunction with this unit showed that larval control could be obtained for protracted periods by treating breeding areas with dieldrin or heptachlor (Goulding *et al.* 1953, Keller *et al.* 1954, LaBrecque 1954).

Utilizing the results of these studies, the Palm Beach County Mosquito Department obtained satisfactory control of sand fly larvae for 2 to 3 years with several aerial applications of dieldrin granules. The first large scale airplane treatment, using 5% granules, was made in the Hypoluxo mangrove marshes in 1954. This area, which consisted of approximately 200 acres, was treated at the rate of 20 lb. to an acre. Thereafter, 10% granules were used at the rate of 10 lb. to an acre. The first treatments gave excellent control of *C. furens*, but not of *C. melleus* (Coq.), the yellow sand fly. This species was found breeding in untreated strips of sandy shore line where tidal fluctuations occurred. When these strips were treated *melleus* was also controlled. By the end of 1957 annoyance from *furens* was increasing, and it appeared that the efficacy of dieldrin was decreasing. An aerial application of heptachlor impregnated granules in the fall of 1957 gave little if any control.

During the summer of 1958 laboratory studies were conducted to determine whether larvae from this area were resistant to various insecticides. Samples of soil containing sand fly larvae were collected from marshes in Palm Beach and St. Lucie Counties. The marsh in Palm Beach County had been treated three times with dieldrin (April 1954, May 1955, and April 1957), once with malathion (July 1956), and once with heptachlor (November 1957), each time at the rate of 1 lb. per acre. The marsh in St. Lucie County had never been treated for sand fly control and served as a measure of normal susceptibility of *furens* larvae. The larvae were recovered from the soil samples by the method developed by Bidlingmayer (1957). The samples were accumulated in a quart can until the can was about three-fourths full. About 2 inches of dry beach sand was poured over the marsh soil and the can was filled with water. The next day the sand, into which the larvae had then moved, was transferred to a pudding pan and washed, and the wash water was poured through a 200-mesh screen. The residue on the screen was washed into a black photographic tray, 12×10×2 inches. The whitish larvae were easily seen and collected as they swam across the dark bottom of the tray.

For testing, 25 larvae were transferred with a medicine dropper into a beaker containing 5 ml. of water, which subsequently was added to 95 ml. of a suspension or solution of the larvicide made by adding an acetone solution to water. After 24 hours the contents of each beaker were poured into a black tray and the dead larvae were counted. Larvae were considered dead if after considerable prodding they did not swim with their characteristic whiplike movement. The instar of the test larvae was unknown; however, only larger larvae of approximately the same size were used.

The results are given in table 1. Larvae from the Palm Beach County marsh were more than 100 times as resistant to dieldrin, heptachlor, chlordane, and lindane and about 10 times as resist-

[1] Accepted for publication January 16, 1959.
[2] Entomology Research Division, Agr. Res. Serv., U.S.D.A.
[3] Director of the Palm Beach County (Florida) Mosquito Department.
[4] F. W. Harden, director of the St. Lucie County Sanitary District, and W. C. Byrum of the Palm Beach County Mosquito Department, assisted in these studies.

Table 1.—Susceptibility of *Culicoides furens* larvae from treated and nontreated Florida marshes to various larvicides. (2 tests.)

LARVICIDE	PER CENT MORTALITY IN 24 HOURS AT INDICATED CONCENTRATION (P.P.M.)							
	50	20	10	1	0.1	0.01	0.001	0.0001
	St. Lucie County (nontreated)							
Dieldrin	—	—	—	100	100	94	62	12[a]
	—	—	—	100	100	74	2	0
Endrin	—	—	—	100	100	87	0	0
Lindane	—	—	—	100	100	54	0	0
Chlordane	—	—	—	100	100	52	0	0
DDT	—	—	—	100	100	22	0	0
Parathion	—	—	—	100	100	24	0	0
Malathion	—	—	—	100	44	0	0	0
Heptachlor	—	—	—	100	20	34	0	0
	—	—	—	100	0	2	0	0
Bayer 21/199	—	—	—	100	12	0	0	0
	Palm Beach County (treated)							
Dieldrin	—	—	—	54	0	0	0	0
	100	25	23	4	0	—	—	—
	100	84	77	0	0	—	—	—
Endrin	—	—	100	100	78	4	0	—
Lindane	—	—	100	63	6	0	0	—
Chlordane	—	—	64	72	0	7	0	—
DDT	—	—	100	100	94	38	2	—
Parathion	—	—	—	100	100	17	0	0
Malathion	—	—	—	100	86	9	0	0
Heptachlor	—	—	41	8	0	2	0	—
Bayer 21/199	—	—	—	100	35	0	0	0

[a] 100% Mortality at 0.05, 88% at 0.005, and 48% at 0.0005 p.p.m.

ant to endrin as those from St. Lucie County. Although the Palm Beach marsh had not been treated with chlordane, lindane, or endrin, cross-resistance to these materials was evident. No resistance to DDT or the organophosphorus insecticides—parathion, malathion, and Bayer 21/199—could be demonstrated. Dieldrin was the most effective larvicide against the normal strain, whereas parathion, DDT, and malathion were highly effective against the resistant strain.

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Some Sugars Secreted by the Woolly Alder Aphid[1]

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Honeydew from the woolly alder aphid, *Prociphilus tessellatus* (Fitch), feeding on silver maples, *Acer dasycarpum*, was collected from a parked car at Dobson, N. C. June 1958. Identity of the sugars present was made by Dr. J. W. White, Jr., Head, Honey Investigations, Plant Products Laboratory, Philadelphia, Pa., based on the chromatographic movement compared with known sugars, enzyme reactions, and color reactions on papergrams.

The sample appeared to have glucose, fructose, sucrose, maltose, erlose, glucosyl erlose, maltosyl erlose, maltotriosyl erlose, an indefinite number of higher members of this series, maltotriose, maltotetraose, maltopentoase and higher members of this series also. It showed at least six amino acid-type spots which we have not identified, inorganic phosphate, and at least one organic phosphorous-containing compound.

Dr. J. W. White, (1958) stated that they were not absolute identifications but that there appear to be two types of honeydew, the melezitose and the glucosucrose (erlose) type. Each of these trisaccarides is elaborated from sucrose by an enzyme system in the insect involved.

Goldschmidt & Burkert (1955) concluded that erlose is formed as a secondary product by transglucosidation in the body of the bee. The findings presented here indicate that erlose is present in honeydew secreted by *Prociphilus tessellatus*.

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Control of the Bagworm with Dibrom[1]

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A spray material[2] containing dimethyl 1, 2 dibromo 2,2 dichloroethyl phosphate (Dibrom®) proved to be highly toxic to the bagworm, *Thyridopteryx ephemeraeformis* (Haw.) in tests conducted in 1957 and 1958.

The tests in 1957 were made with an emulsifiable concentrate containing 4 pounds of Dibrom per gallon and labeled RE-4355. Applied to arborvitae and juniper on July 9, when the bags were about one-half inch long, RE-4355 at rates of ½, 1, and 2 pints per 100 gallons of water all gave a 100% kill. At rates of ⅛, ¼, ½, and 1 pint per 100 gallons of water applied July 15, the two higher rates gave 100% kills, but the two lower rates gave incomplete kills. A complete kill was obtained with 1 pint of RE-4355 per 100 gallons of water applied to a blue spruce, July 22 when the bags were about 1 inch long. On August 5, when the bagworms were almost mature, a 100% kill was obtained with 2 quarts of RE-4355 per 100 gallons of water, but on August 21, with 50% of the bagworms pupated, RE-4355 at the same rate killed only 44.4% of the insects. In 1958 a complete kill was obtained with "Dibrom 8 Emulsive" at the rate of 1 quart per 100 gallons applied to heavily infested Pfitzer junipers on August 19. At that time, 10% of the worms (probably all males) had reversed in the bags and 80% appeared to have stopped feeding.

The action of Dibrom is very fast, a matter of minutes on worms in bags one-half inch and less in length, but on large nearly mature bagworms, 1 or 2 days may pass after spraying before all movement ceases.

The residual action of Dibrom appears to be short. No kill was obtained when bagworms were caged on plants July 17, that had been sprayed 8 days previously with RE-4355 at rates of ½, 1, and 2 pints per 100 gallons of water. This indicates the need for thorough and complete spray coverage.

The Dibrom sprays gave no evidence of phytotoxicity to any of the plants treated.

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[2] Supplied by California Spray-Chemical Corporation.

Preassay Activation of Thimet[1]

R. MISKUS AND S. HASSAN[2]

Many of the new insecticides used on agricultural crops today are active due to their cholinesterase inhibiting properties. Since in many cases, no satisfactory chemical method of analysis is available for determining residues of these compounds, use of this cholinesterase inhibition property has been made for a method of analysis (Giang & Hall 1951). However, some of these insecticides are not strong cholinesterase inhibitors *in vitro* (Metcalf *et al.* 1957). Therefore some means of activation has been used to increase inhibitory action so that more sensitivity can be achieved in residue analyses as was the case with Bayer 19639 (Miskus *et al.* 1959) and with work carried out by Cook *et al.* (1958), Bowman & Casida (1957), and Metcalf *et al.* (1957).

When the procedure used in analysis for Bayer 19639 was applied to Thimet, the results were not satisfactory and modifications were found to be necessary. Since it has been shown that oxidation of these cholinesterase inhibitors in many cases increases their inhibiting powers, Thimet was oxidized with peracetic acid in an effort to increase its action over that which it exhibits unmodified (Metcalf *et al.* 1957, Cook 1958). The changes that were made in the procedure used for the analysis of

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Bayer 19639 in order to adapt the method for Thimet are discussed herein.

First, the oxidation carried out with a 1–5 mixture of 30% hydrogen peroxide and glacial acetic is performed in chloroform rather than benzene, since it was found that some of the oxidized products of Thimet did not partition in favor of benzene, but stayed in the water phase. Secondly, shaking at 2-minute intervals during the 10-minute oxidation is carried out since the chloroform phase is now below. Thirdly, the temperature of the water bath was reduced to 60° C. so that losses of volume would be less likely to occur during the oxidation.

Figure 1 shows the effect of time of oxidation of Thimet upon the 50% cholinesterase inhibition value. As can be seen in figure 1, the 10-minute time gives the optimum activity for Thimet and this period is used for preassay activation. Table 1 shows analytical recovery data for Thimet added to three different plant extracts.

An alternative method for changing inhibition values for oxidized Thimet would obviously be that of temperature variation for the oxidation, but this was not carefully determined. Our experience also indicates that erratic recovery results and change in 50% inhibition values occur as the peroxide ages, but a change to fresh peroxide corrects variation, although the exact reason has not been determined.

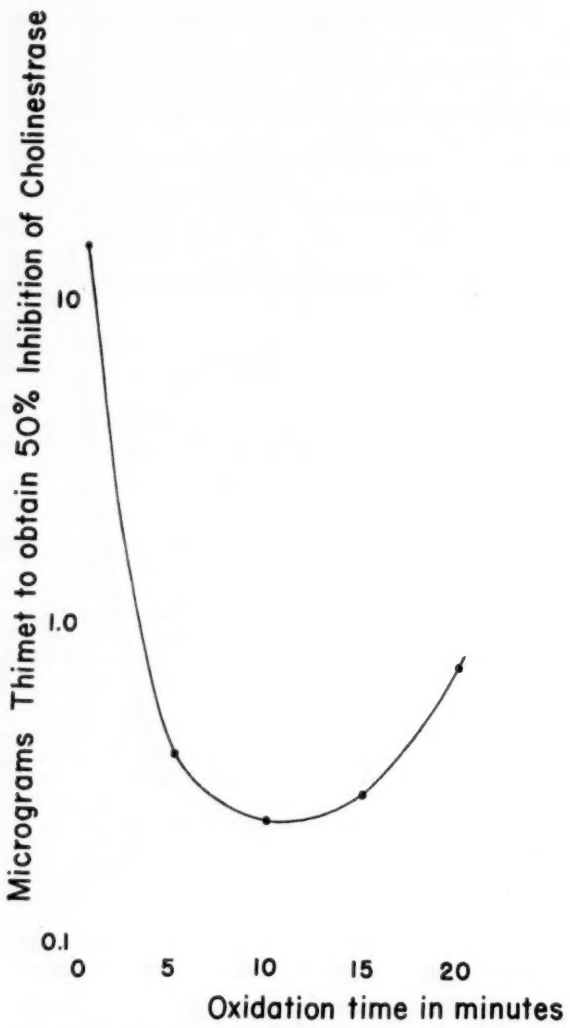

FIG. 1.—Effect of time of oxidation of Thimet upon 50% inhibition values.

Table 1.—Recovery data for Thimet added to 1.0-gram aliquots of plant extracts.

Crop	Thimet (p.p.m.) Added	Found	Per Cent Recovery
Cabbage	0	0.27	—
	1.0	1.05	78
	2.0	2.15	94
	0	.22	—
	1.0	.90	70
	2.0	2.3	104
Strawberry	0	.31	—
	1.0	1.45	114
	2.0	2.26	98
	0	.28	—
	1.0	1.35	107
	2.0	2.6	116
Apple	0	.42	—
	1.0	1.45	103
	2.0	2.26	92
	0	.28	—
	1.0	1.1	82
	2.0	2.0	86

Since figure 1 shows that with time, Thimet seems to undergo loss of inhibitory power under the oxidation conditions used, work was carried out to determine the effect of the oxidation procedure on the proposed metabolites of Thimet (Metcalf *et al.* 1957). Table 2 shows the inhibitory values of Thimet and metab-

Table 2.—Fifty per cent inhibition values for Thimet and metabolites before and after oxidation.

Compound	I_{50} (in Micrograms) Before Oxidation	After Oxidation
$(C_2H_5O)_2P(S)SCH_2SC_2H_5$	75	0.9
$(C_2H_5O)_2P(S)SCH_2S(O)C_2H_5$	1.5	.9
$(C_2H_5O)_2P(S)SCH_2S(O)C_2H_5$	.76	.55
$(C_2H_5O)_2P(O)SCH_2S(O)C_2H_5$	.59	.25
$(C_2H_5O)_2P(O)SCH_2S(O)C_2H_5$	.13	.18

olites before and after oxidation. It can be seen that under the conditions that would be used for the residue analysis, there is very little loss of inhibitory power of any of the metabolites.

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Effect of Several Pesticides on the Germination of Three Varieties of Flower Seeds[1]

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Mites consistently infested only three varieties of flower seeds in a seed producer's warehouse in Santa Barbara County, California, although more than 1100 varieties of flower seeds were in storage annually. The presence of mites in large numbers has been objectionable to seed buyers, and workers in the seed plant have complained of undue irritation after cleaning infested seeds. The use of residual pesticides for the elimination of mites was indicated, but the effect of candidate materials on the germination of seeds was unknown. It is the purpose of this note to summarize results from germination tests with three varieties of flower seeds after treatment with several pesticides at dosages somewhat higher than those normally considered practical for use.

MATERIALS AND METHODS.—The varieties and moisture content of flower seeds used were: *Centaurea Cyanus* (Bachelors-button, double-choice mixed)—6.9%, *Iberis umbellata* (Candytuft, choice mixed)—6.9%, and *Alyssum procumbens* (Carpet of Snow)—6.6%. The moisture contents were determined by drying approximately 2 gms. of seeds in a mechanical-convection oven for 1½ hours at 130° C. ± 2° C. and calculating the per cent loss in weight. Three moisture determinations were made for each variety.

Undiluted wettable powder formulations of pesticides applied to seeds were as follows:

Treatment No.	*Pesticide*	*Concentration of Pesticide (%)*
1	Aramite	15.0
2	Chlorobenzilate	25.0
3	Kelthane	18.5
4	Ovex	50.0
5	Tedion	25.0
6	Malathion	25.0

Pesticides were applied at the rate of 1½ gm. of wettable powder to 15 gm. of seeds. Treated seeds were thoroughly blended and held in sealed glass jars at room temperature (65° to 90° F.). All treatments were replicated three times, including untreated control samples for each variety.

Germination tests were made at intervals of 1 week, 3 months, and 6 months after treatment. Seeds were screened to remove as much wettable powder as possible before planting. The remaining seeds and pesticide were reblended, sealed in glass jars, and held for the next germination test. Germination tests were made in accordance with regular methods of Official Seed Analysts (see p. 59–60, Assoc. Offic. Seed Analysts 1954).

Table 1.—Mean variations in the percentages of germination of treated seeds from untreated control samples of three varieties of flower seeds.

PESTICIDE TREATMENT NO.	TIME AFTER TREATMENT		
	1 Week	3 Months	6 Months
	Centaurea Cyanus		
1	+1	−1	−4
2	+4	−5	−5
3	+4	−1	−2
4	0	−3	0
5	+2	−1	+2
6	+1	−4	−3
	Iberia umbellata		
1	0	−2	−1
2	+1	−1	+3
3	−1	−5	+2
4	+3	−2	−2
5	−7	+2	−3
6	+2	−9	−3
	Alyssum procumbens		
1	−6	+4	+3
2	−6	+2	−2
3	0	+4	+5
4	−5	+3	+3
5	+1	+2	+1
6	−6	+2	−1

RESULTS.—The percentages of germination was the only criterion recorded for comparison of results. Normal seedlings, those with all essential plant parts, were counted as germinated. Results are summarized in table 1 as the mean variations in the percentages of germination of treated seeds from untreated control samples. The results indicate that the pesticides tested may be applied to the three varieties of flower seeds for the control of mites without seriously decreasing germination.

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[1] Accepted for publication November 15, 1958.

Potato Aphid Control on Tomatoes[1]

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The potato aphid, *Macrosiphum solanifolii* (Ashm.), is primarily a pest of potatoes but at least four publications have appeared between 1917 and 1958 that have described detrimental infestations of this pest on tomatoes, House *et al.* (1917), Smith (1919), Taschenberg (1949), and Taschenberg (1955). The most recent insecticide evaluation studies by Taschenberg (1955) showed that demeton (Systox), parathion, malathion, and EPN were effective in reducing the aphid infestations. The object of the present study was to evaluate the more recently developed insecticides against the potato aphid attacking tomatoes in Texas. An insecticide of low mammalian toxicity that would ef-

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fectively reduce an aphid infestation, retain some residual qualities, and be available in the area would be welcomed by the vegetable grower.

MATERIALS AND METHODS.—This test was carried out during April 1958. The tomato plants were planted on beds for furrow irrigation with 40-inch centers, averaged 10 inches in height, and nine compound leaves per plant when treated. Sprays were applied in the evening when there was no appreciable wind movement.

The design was a randomized block having each treatment replicated four times on experimental plots 2 rows wide and 25 feet long with 5-foot alleyways. The effectiveness of the treatments was evaluated in the field by counting live aphids on five randomly selected leaves per plot at 1-, 4- and 7-day intervals following application. Average leaflets per coumpound leaf were 4, 5, and 7 at these intervals. The number of aphids per plant in the check plots are given in table 1. Data were analyzed for sources of variance by applying the F test and a multiple range test outlined by Duncan (1955) to evaluate differences between treatment means.

Table 1.—Effectiveness of several insecticides against the potato aphid on tomatoes, 1958.

TREATMENT 1/ACRE	PER CENT CONTROL[a] FOLLOWING APPLICATION		
	1 Day	4 Days	7 Days
Sevin	92.9	88.7	28.6
Ronnel	76.8	96.9	62.1
Diazinon	92.9	97.9	59.7
Malathion	92.9	74.2	28.6
Trithion	67.9	94.9	72.6
Perthane	58.9	19.6	0
Dibrom	100.0	69.1	48.4
Untreated[b]	11.2	19.4	49.6

[a] Based on the untreated control plots.
[b] Mean number of aphids per leaf.

Treatments were applied to the staked row of each plot. Each insecticide was applied at the rate of 1 pound active ingredient per acre in spray form. Two nozzles per row were used and directed to hit the underside of the leaves. Forty pounds of pressure per square inch delivered 20 gallons of liquid per acre.

RESULTS AND DISCUSSION.—Table 1 gives the results of these treatments. Sevin®, 1-naphthyl *N*-methylcarbamate, Diazinon, malathion, and Dibrom® (*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate) had the greatest initial toxicity but Sevin and malathion soon became less effective. Ronnel (formerly known as Dow ET-57, Korlan, or Trolene) and Trithion appear to be slow in action but have a greater length of insecticidal activity as illustrated at the 4- and 7-day readings. Statistical analysis indicated that there were no differences among the treatments 1 day after application. Four days after treatment all the chemicals were effective except Perthane which was ineffective. On the seventh day after treatment Trithion, ronnel, Diazinon and Dibrom gave superior control to Sevin, malathion, and Perthane, but no differences were indicated between these chemicals. No phytotoxic symptoms were noted in the treated plots.

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Autogeny in *Aedes taeniorhynchus* (Wied.)[1]

ARDEN O. LEA and PATRICK T. M. LUM

As Spielman (1957) points out, the phenomenon of autogeny in mosquitoes is not uncommon. Those species reported in the literature as being autogenous have been listed by Spielman (1956), while two recent additions are: *Culex tarsalis* Coq. reported by Bellamy & Kardos (1958) and Chao (1958), and *Wyeomyia smithii* (Coq.) observed by Price (1958).

Lacking data to the contrary, a salt-marsh mosquito *Aedes taeniorhynchus* has been considered to be anautogenous. However, during recent laboratory studies on feeding habits and ovarian development, it was found that some *taeniorhynchus* females do develop a small initial batch of eggs without a blood meal. Generally, this autogenous egg batch contained 10 to 20 eggs but in some individuals was as high as 35 to 40 eggs.

In order to determine whether autogeny was of general occurrence in nature, biting females collected in the field were allowed to feed on a rabbit and induced to oviposit. The eggs were then hatched, the larvae reared on dog food, and the adults held without access to blood for 5 to 7 days before the amount of ovarian development was determined. Autogenous individuals were found among the resulting females, as well as among adults which emerged in the laboratory from field collections of larvae and pupae. Autogeny was found to occur in seven different areas of the salt-marsh in Indian River County, Florida. Collections of biting females from Virginia and Biscayne Keys near Miami, from Royal Palm ranger station in the Everglades National Park, and from Bradenton, Florida showed that autogeny is present in those localities and presumably occurs in the intervening areas throughout the state.

Although it is not known whether interbreeding takes place, both autogenous and anautogenous forms occur together in the same areas, since they have been reared from the same sod sample, in addition to the above mentioned collections of biting females.

Autogeny in *Aedes taeniorhynchus* is of considerable interest because it adds a new facet to any study involving the female of this species. With at least two physiologically different forms in the breeding area, it is important to learn in what ways this difference is reflected in the mosquitos' behavior. One point in particular which should be determined is whether autogenous females participate in the migration flight of recently emerged females (Nielsen 1958), and if so, whether they drop out of the migration soon after taking off, thus conserving their food reserves and tending to remain closer to the breeding areas than anautogenous migrants.

An attempt is being made to establish a colony of autogenous and one of anautogenous *taeniorhynchus* using the methods outlined by Haeger (1958) for this species. Although this work is hampered by the difficulty encountered in inducing mating in the laboratory, viable eggs have been obtained. Once these colonies can be maintained, experiments on their biology and behavior will be feasible.

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The Influence of Two Insecticides on the Emergence of *Lyctus planicollis* LeC.[1]

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Few laboratory techniques for studying the effectiveness of insecticidal solutions against the southern lyctus beetle, *Lyctus planicollis* LeC., or other species of powder-post beetles are mentioned in the literature. Smith (1956) determined the influence of several oil preparations and solvents against *L. planicollis*.

The following procedure was used to compare the insecticides, chlordane and dieldrin, and their inert ingredients as soaking for controlling laboratory-induced infestations of *L. planicollis*.

Heat-dried red oak wood, with a high starch content, was used in this test. Wood billets, 6 inches long by approximately 1½ to 2½ inches in diameter, were split into triangular sections leaving two inner flat surfaces and one outside rounded edge exposed. Other billets, 6 inches long by approximately 1 to 2 inches in diameter, were split into halves leaving one inner flat surface and one outside rounded surface exposed.

Forty of the 6-inch triangular wood sections and 40 of the 6-inch half-round wood sections were cut into 3-inch samples after remaining in the laboratory for about 6 months. Both halves of each 6-inch section were weighed and placed in one Ball No. 808 glass. A layer of sand, about ¼ inch deep, was put on the bottom of the glass. Initial infestation of the wood samples was accomplished by placing five females and five males, less than 3 days old, on the wood samples in the glass and fastening a muslin cloth over the mouth of the glass with a rubber band. The beetles and sand were removed after the beetles died.

Following the death of the adults the two 3-inch samples of wood were placed in separate glasses and were thereafter considered as a group. Approximately 2 months after the initial exposure of the wood samples to beetles, they were examined for the presence of powder. If powder was observed the wood was considered infested with beetles. All of the original wood groups, except two, were infested and these were replaced with two additional wood groups cured and exposed to beetle attack at the same time and in the same manner.

Prior to chemical treatment of the wood samples, melted paraffin was applied to the two ends of each wood sample to prevent longitudinal penetration of the chemicals. The amount of paraffin adhering to each wood sample was determined by weighing the wood sample immediately before and immediately after the paraffin was applied.

Each half-round and each triangular group was allocated to one of the following treatments: 2% chlordane emulsion, 0.5% dieldrin emulsion, inert ingredients of the chlordane emulsion, inert ingredients of the dieldrin emulsion and water. There were five replicates of each treatment. Fifteen of the half-round and 15 of the triangular groups were left as untreated checks.

The wood samples were chemically treated to control the beetle infestations 2½ months after the samples were initially exposed to adults of *L. planicollis*.

The two infested 3-inch samples of wood in each group were placed in separate glasses and the treatment emulsion randomly assigned to the group was added until it completely filled the glasses. A metal cover, when placed over the rim of the glass, prevented the wood sample from floating to the top of the liquid.

After complete immersion of the wood samples for 2 hours, they were removed from the liquid and allowed to drain. The two samples in each group were placed in individual clean glasses. Thereafter, bimonthly inspection of the wood samples disclosed the number of adults which had emerged from the wood and the beetle activity in the wood (indicated by powder on the surface of the wood or on the bottom of the glass). After each inspection all powder was removed from the glass and wood sample. Observations were continued for 1 year from the initial exposure of the wood group to adults. The wood samples were weighed at the completion of the 1-year test period and the final weights were compared with their original weights.

Soakings of half-round and triangular wood samples for 2 hours in 2% chlordane emulsion or 0.5% dieldrin emulsion gave complete control of populations of *L. planicollis* (table 1). Soaking half-round and triangular wood samples in an emulsion of the inert ingredients of the chlordane formulation produced means of 4.0 and 13.0 adults per sample, respectively, whereas other samples soaked in an emulsion of the inert ingredients of the dieldrin formulation produced means of 11.4 and 10.6 adults per sample, respectively. The half-round and triangular wood samples soaked in water and those not treated yielded a larger number of adults. The ratio of males to females was similar in all of the treatments and the untreated check.

There is a direct correlation, significant at the 1% level, between the number of emerging adults and the weight loss of the triangular wood samples. No correlation of adult emergence to weight loss of the half-round wood samples could be shown; however, this failure was traceable to two wood samples which had a very high per cent weight loss. Beetle activity was extremely heavy in these two samples and large quantities of

[1] Accepted for publication January 19, 1959.

[2] Now with the Wilson Exterminating Company, Winston-Salem, North Carolina.

Table 1.—Mean number of adults of *Lyctus planicollis* that emerged from treated wood samples and the loss or gain in weight of the samples in each group.

Wood and Treatment[a]	No. of Beetles		Per Cent Weight Loss or Gain	
	Range	Mean	Range	Mean
Half-round				
a	0	0	+ 1.3 to +3.6	+ 1.94
b	0	0	+ 0.7 to +2.6	+ 1.18
c	0– 9	4.0	− 1.6 to +1.6	+ 0.26
d	3– 26	11.4	− 7.7 to +1.4	− 2.16
e	3– 98	41.4	−58.0 to −0.3	−17.22
f	15–102	41.1	−40.2 to −1.9	− 9.00
Triangular				
a	0	0	− 1.1 to +2.1	+ 0.78
b	0	0	0 to +3.2	+ 1.26
c	0– 24	13.0	− 7.6 to +1.0	− 3.0
d	0– 17	10.6	− 4.8 to +2.2	− 0.82
e	41– 65	46.6	− 7.5 to −4.1	− 6.08
f	0– 69	34.6	−22.5 to +0.2	− 6.60

[a] a, chlordane emulsion, 2%. b, dieldrin emulsion, 0.5%., c, inert ingredients of the chlordane emulsion. d, inert ingredients of the dieldrin emulsion. e, water. f, untreated check.

powder were "pushed" from the wood by larval activity, thus reducing the weights of the two wood samples. The adult emergence and the weight loss of the half-round samples, other than that of the two samples mentioned above, indicated a correlation between the two factors.

Analysis of the weight loss of wood samples in relation to the treatments and the untreated check showed no differences, because of excessive weight losses in the two wood samples mentioned in the previous paragraph. However a gain in weight occurred in wood samples soaked in chlordane or dieldrin emulsions. This gain was probably due to one or more of the following: the residue of toxicant left on or in the wood samples, the residue of the inert ingredients, or an increase in the moisture content of the wood samples. Some wood soaked in emulsions of the inert ingredients showed a weight gain, whereas others showed a weight loss. Wood samples soaked in water or left untreated showed mean weight losses much greater than those found in the other treatments. These losses were due to heavy larval activity and the "pushing" of powder from the wood samples.

Reference Cited

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Effects of Chicken Body Louse Infestation on Egg Production[1]

Elmer E. Gless[2] and Earle S. Raun, *Department of Zoology and Entomology, Iowa State College, Ames*

Louse control in the laying flock has always been a problem for poultrymen. In the past many publications have been concerned with the control of ectoparasites, but few workers have attempted to determine the actual extent of the damage they cause. Roberts & Smith (1956) estimated the annual loss caused by ectoparasites to the poultry industry at 80 million dollars. A report published in 1948 by Warren *et al.* suggested that the common chicken body louse did not reduce the number of eggs laid by an infested hen. Edgar & King (1950) found differences in weight of the hens as well as in numbers of eggs produced after an 11-month experiment involving moderately heavy infestations of the louse.

The purpose of this study was to determine the actual extent of the damage incurred by heavy infestations of *Menacanthus stramineus* (Nitz). The experiment was designed to analyze any reduction in numbers and weight of the eggs caused by the louse infestation.

Methods and Materials.—The poultry house used in this experiment measured 20 by 40 feet with single walls and concrete floor. Twenty-four cages measuring 3 feet wide, 4 feet long, and 3 feet high were constructed within the building. Each cage was equipped with a roost, a roll-away nest box, water and oyster-shell containers, and a hanging-drop feeder. The feed was a standard laying mash supplied by the local elevator. Lights were installed and timed for a 13-hour day.

On October 1, 1957, five birds were placed in each cage. Of these, three were type A-1 and two type B-1 genetic inbreds from the Ames-In-Cross Hatchery at Roland, Iowa.

Production records were kept for 4 weeks and the hens were then moved in order to pair pens of like production. Random selection of location and infestation was made with the flip of a coin. Two weeks later 10 *Menacanthus stramineus* were placed on each of the birds in the 12 cages of birds to be infested. Egg numbers and weights were recorded in the new location for the ensuing 14 weeks.

Since infested and louse-free birds were in adjoining pens frequent inspections for lice were made of louse-free birds. In two instances a few lice were seen on birds to be kept louse free. In both cases pyrethrum dust was applied by the pinch method to eliminate these lice.

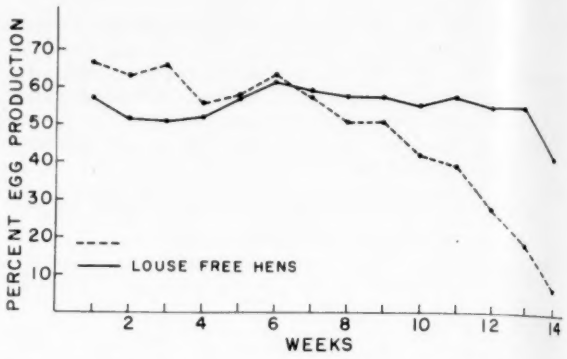

Fig. 1.—Percentages of egg production per week for louse-free and louse-infested hens.

Results and Conclusions.—The percentage of egg production was calculated on the hen-day basis because two birds died in each treatment at different times. Table 1 shows the average per cent production and the F value from the analysis of variance for each week for both infested and uninfested birds. Differences were not significant until the 10th week after infestation.

An analysis of the egg totals for the entire 14 weeks after infestation indicated that the differences in production between infested and uninfested birds was significant at the 99% level.

Since the louse population was not large until about the fourth week, an analysis of variance was made of the production for the last 10 weeks. The production differences due to the louse population were significant at the 99% level for this period.

The graph (fig. 1) taken from the data presented in table 1 indicates the percentages of production by weeks after infestation with the chicken body louse, *Menacanthus stramineus*. For the entire 14-week experiment, egg production by the infested hens was reduced 15%. During the last 10 weeks, egg production was reduced 26%. Production during the 14th week was reduced 84%.

The analysis of the data indicated that there were no differences in weights of the eggs due to the louse infestation.

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[2] Now Entomologist, Entomology Research Division, Agric. Res. Serv., U. S. Department of Agriculture, Kerrville, Texas.

Table 1.—Percentages of egg production for louse-free (treatment 1) and louse-infested (treatment 2) chickens by week.

Week	Per Cent Production		F Value[a]
	Treatment 1	Treatment 2	
1	57.21	66.67	3.54
2	51.55	62.38	3.60
3	50.60	65.91	1.61
4	52.06	55.48	.52
5	56.67	56.43	.0015
6	61.67	62.86	.005
7	59.11	57.98	.02
8	57.80	50.78	1.83
9	57.80	50.72	1.49
10	55.20	42.20	4.96*
11	57.50	39.35	18.50**
12	55.12	28.15	51.24**
13	55.48	18.35	35.47**
14	42.03	6.67	26.55**

[a] Tabular $F_{.05} = 4.84$ at 1 and 11 d.f.: $F_{.01} = 9.65$ at 1 and 11 d.f.

At the termination of the test two infested hens were taken into the laboratory, placed in a tight paper-lined box, and dusted with lindane. The lice and the lindane dust were gathered from the bottom of the box and the lice separated from the lindane and counted. There were 24,627 lice removed from one hen and 21,500 removed from the other.

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Invasion of the Southeast by a Western Leafhopper, *Homalodisca insolita*[1]

H. N. POLLARD, W. F. TURNER,[2] and G. H. KALOOSTIAN,
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A leafhopper whose known range until 1950 was Texas, Mexico, and Arizona has invaded the Southeast and established itself as the dominant species of the tribe Proconiini in its new habitat. Since its discovery in the vicinity of Fort Valley, Ga., in 1950, *Homalodisca insolita* (Walker) has been incriminated as a vector of the phony peach disease virus (Turner & Pollard 1955). Although an efficient experimental vector of this virus, its food and flight habits preclude its becoming an important natural vector.

The discovery of *insolita* in the Southeast and its spread to and throughout that area presents an intriguing problem of insect movement. It is difficult to determine whether the insect moved across the continent gradually or in long hops. In the movement it faced tremendous climate changes and, in the Southeast, shifted to a secondary host plant .In Texas its preferred host is millet (*Panicum texanum* Buckl.); in its new habitat it is most commonly found on Johnson grass (*Sorghum halepense* (L.)).

The original two specimens of *insolita* were taken in the Southeast on sticky-board traps (Kaloostian & Yeomans 1944) exposed on the grounds of the U. S. Horticultural Field Laboratory near Fort Valley, Ga. These insects, which resembled *H. coagulata* (Say), an important natural vector of the phony peach virus, in size and shape but not in color, were entirely new to us after 15 years of intensive surveying and trapping. D. A. Young, of the former Bureau of Entomology and Plant Quarantine, identified the leafhoppers as *Homalodisca insolita* (Walker), a species closely related to the vectors of phony peach (Turner 1949), with a notation that the only specimens in the National Museum collection were from Texas and Mexico, and that Melichar (1924) listed Texas, Mexico, and Arizona as the known range of the species.

FIG. 1.—New area occupied by *Homalodisca insolita*.

Table 1.—Numbers of *Homalodisca insolita* collected on sticky-board traps at different levels on poles, 1950–57, Fort Valley, Ga.

YEAR	2 FEET	6 FEET	10 FEET	TOTALS
1950	1	1	—	2
1951	9	—	—	9
1952	105	5	—	110
1953	430	7	4	441
1954	234	19	6	259
1955	143	5	3	151
1956	315	9	3	327
1957	568	22	8	592
Total	1,805	68	24	1,891
Per cent of total	95.4	3.6	1.0	100

Since its discovery in Georgia this species has been found in eastern Texas, Louisiana, Arkansas, Tennessee, Mississippi, Alabama, Florida, and North and South Carolina (fig. 1). Population increase at the original point of discovery, as indicated by sticky-board trap collections from 1950 through 1957, is summarized in table 1.

For general insect survey and detection purposes, sticky-board traps are suspended from limbs of trees. However, the trap installations at the Fort Valley laboratory (fig. 2) were specifically constructed for studying the direction and pattern of flight at

[1] Accepted for publication January 28, 1959

[2] Retired May 31, 1957.

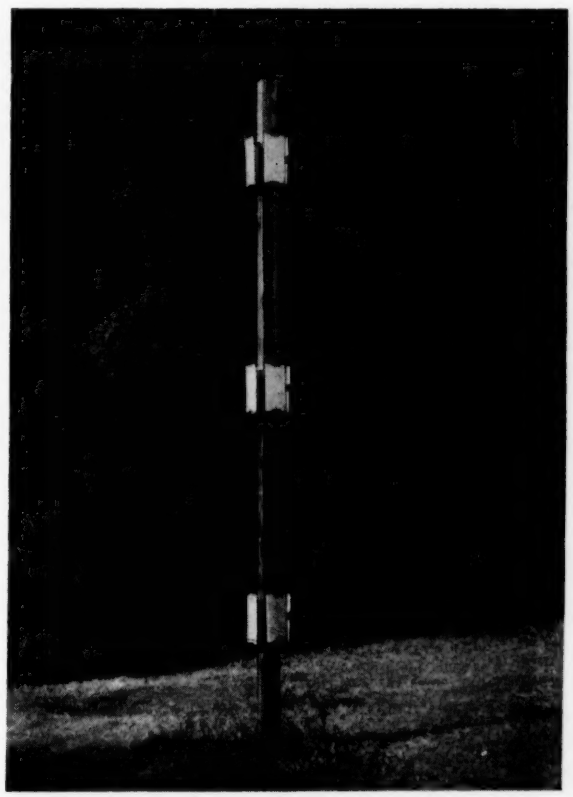

FIG. 2.—Sticky-board traps exposed on a pole for studying flight habits of *Homalodisca insolita*.

three altitudes. In January 1950 six poles were installed around a small wood lot of deciduous trees near the laboratory. On each pole 12 traps were exposed in such a manner that three traps faced in each direction and there were four traps each at 2, 6, and 10 feet above the ground. The new arrival was detected October 5, 1950 on traps exposed since September 29. As the traps were continuously operated, attempts were made to pin-point the infestation by sweeping the vegetation near the traps, and by beating the shrubs in and near the woods. These efforts were unsuccessful until July 12, 1952, when an adult was swept from ragweed. Two months later the traps indicated an explosive increase in population, and soon thereafter the leafhop ers were readily swept from Johnson grass. A close examination revealed the presence of immature stages of the insect on this grass.

Our observations suggest that we experienced the initial invasion, establishment, and dispersion of an important species in a local area. It is assumed that the establishment of the insect at Fort Valley was one of the small units of a general invasion as the insect progressed from west to east. Surveys made over an expanding area, as time and circumstances permitted during the last 7 years, indicate that *insolita* is well established in the Southeast.

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Effectiveness of Sevin Against the Grey Garden Slug[1]

ROBERT F. RUPPEL[2]

The grey garden slug, *Deroceras reticulatum* (Muller), is a pest in many horticultural and ornamental crops and in some pasture and field crops in the cooler regions of Colombia. In previous studies of chemicals for the control of this pest (Ruppel & Posada, unpublished), the carbamates Pyrolan, Pyramat, and maneb were among the better materials tested. As Pappas & Carman (1955) also found the carbamates Isolan, Pyrolan, and G341 [1-ethyl-3-methylparasolyl-(5)-dimethyl carbamate] effective against the brown garden snail, *Helix aspersa* Muller, this class of poisons seems to be promising for use against the mollusca. Since Sevin® (1-naphthyl *N*-methylcarbamate) is a new insecticide of this class that has a low mammalian toxicity and is relatively nonphytotoxic, a test of Sevin as a bait and contact spray poison against the grey garden slug was made at the experiment station "Tibaitatá" near Bogotá during October of 1958.

The baits tested were 1.25% and 2.50% Sevin and a mixture of 1.25% Sevin with 1.25% metaldehyde. The sprays tested were 0.15% and 0.30% Sevin. A bait of a mixture of 7.50% calcium arsenate and 2.50% metaldehyde and a spray of 0.30% metaldehyde were included in the test as standards since these are widely used in control of slugs and were among the best materials noted by Ruppel and Posada. The baits were prepared by grinding wettable powder of 50% Sevin ("Sevin 50W" of the Carbide and Carbon Chemicals Co.) onto wheat bran in a laboratory ball mill. This same powder was used in the spray applications. A 50% concentrate of metaldehyde was made by grinding the technical grade powder with diatomaceous earth. This concentrate was then mixed and ground with the bran and Sevin or a technical grade of calcium arsenate to make the baits, or was reground with diatomaceous earth to make a wettable powder containing 25% of the toxicant for the spray applications.

Test chambers were prepared by placing disks of moistened blotting paper in the bases of petri plates of 10-cm. diameter. The sprays were applied to the interior surface of the covers and bases of the plates at the rate of 1.25 ml. of spray per plate by means of a calibrated spray gun. The baits were mixed with an equal weight of water and 1 gm. of the moist bait was placed in each plate. Untreated bran was placed in the plates that contained the sprays and in the plates used as checks. Three replications were made of each treatment. Ten field-collected grey garden slugs were placed in each plate following treatment. The plates were wrapped in paper to avoid breakage during handling and then stored in a random block arrangement in a shaded portion of an office. At intervals of 24, 48, 72, 96, and 120 hours after the slugs had been placed in the plates, counts were made of the number of slugs in each that showed some response to touch. This criterion for determining "living" slugs was used because it had been found previously that some poisons stupefy the slugs without killing them.

The bait of Sevin mixed with metaldehyde gave a high mortality of the slugs within 48 hours, while the baits of 2.50% Sevin and 1.25% Sevin gave high mortalities of the slugs within 96 and 120 hours, respectively. The sprays of Sevin were ineffective. The spray of metaldehyde was highly effective within 24 hours. The bait of calcium arsenate mixed with metaldehyde was similar in effectiveness to the bait of Sevin-metaldehyde but was somewhat slower in effecting a kill of the slugs. The data obtained in this experiment are summarized in table 1.

Table 1.—Mortality of the grey garden slug obtained with Sevin and some standard treatments.

CHEMICAL	PER CENT CONCENTRATION	TYPE OF APPLICATION	PER CENT MORTALITY AFTER HOURS INDICATED				
			24	48	72	96	120
Metaldehyde	0.30	Spray	87	100	—	—	—
Sevin-metaldehyde	1.25–1.25	Bait	53	97	100	—	—
Calcium arsenate-metaldehyde	7.50–2.50	Bait	33	80	100	—	—
Sevin	2.50	Bait	13	47	60	87	100
	1.25	Bait	3	17	27	30	76
	0.30	Spray	0	0	0	0	3
	0.15	Spray	0	0	0	0	0

From the data presented, it is concluded that Sevin as a bait poison against the grey garden slugs is as effective as the best materials noted by Ruppel and Posada, but is ineffective when used alone in a spray. In work done subsequent to the test reported here, baits of 1.25% to 2.50% Sevin mixed with 2.50% metaldehyde and sprays of 0.25% Sevin mixed with 0.25% metaldehyde proved to be effective against the grey garden slug and also against the greenhouse slug, *Milax gagates* (Drap.). The latter species is common in some regions of Colombia and is much less susceptible to metaldehyde than the grey garden slug. In view of its effectiveness and its safety when compared with the materials currently in use, Sevin appears to be promising for control of mollusca.

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[1] The work reported here was conducted by the Program of Entomology of the Colombian Department of Agricultural Research with the cooperation of The Rockefeller Foundation. Paper No. 101 of the Agricultural Journal Series of The Rockefeller Foundation. Accepted for publication January 28, 1959.

[2] Entomologist with the Colombian Agricultural Program of The Rockefeller Foundation, Bogotá.

Common Names of Insecticides

CARROLL N. SMITH, *Chairman, Committee on Insecticide Terminology*

This revised list of common names of insecticides has been compiled by the Committee on Insecticide Terminology of the Entomological Society of America. It supersedes the list issued in April 1957 and a subsequent addition to this list (Jour. Econ. Ent. **50**: 226–8; **51**: 373), and constitutes the common names to be used in publications of the Entomological Society of America. Like its predecessors, the list is confined to the principal organic compounds that have been developed in recent years, the chemical names of which are too long for convenient use. As in previous lists the chemical definitions and other designations that have been used are shown. These common names, given in the left-hand column of the list, may be used with no explanation as to their chemical composition.

No trademarked names or code numbers are listed as common names. Such designations may be used if the compounds are also identified by proper chemical names, in accordance with the following action taken by the Governing Board at the 1958 annual meeting of the Society: "Until common names of chemical compounds are available to the editors of Society publications, they shall use their well-known editorial prerogatives as to designations employed in Society publications, provided compounds are identified by proper chemical names and the general courtesies of trademarked designations are exercised."

NAME TO BE USED	CHEMICAL DEFINITION	OTHER DESIGNATIONS THAT HAVE BEEN USED
aldrin	not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene	compound 118, Octalene, HHDN
allethrin	3-allyl-2-methyl-4-oxo-2-cyclopenten-1-yl chrysanthemumate	allyl homolog of cinerin I, synthetic pyrethrins
azobenzene	azobenzene	azobenzide, diphenyl diimide
barthrin	6-chloropiperonyl chrysanthemumate	ENT-21557
BHC	1,2,3,4,5,6-hexachlorocyclohexane, consisting of several isomers and containing a specified percentage of gamma	benzene hexachloride, gammexane
butoxy polypropylene glycol	butoxypropanediol polymer	Crag Fly Repellent
chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene	chlordan, Velsicol 1068, Octa-Klor, Octachlor
cyclethrin	3-(2-cyclopenten-1-yl)-2-methyl-4-oxo-2-cyclopenten-1-yl chrysanthemumate	
D-D mixture	mixture of 1,2-dichloropropane and 1,3-dichloropropene	D-D, Dowfume N
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane	chlorophenothane, dichloro-diphenyltrichloroethane
DDVP	2,2-dichlorovinyl dimethyl phosphate	
demeton	mixture of *O,O*-diethyl *S* (and *O*)-2-(ethylthio)ethyl phosphorothioates	a trialkyl thiophosphate, Systox
dicapthon	*O*-(2-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate	Am. Cyanamid 4124
dichloroethyl ether	bis(2-chloroethyl) ether	
dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*-5,6,7,8,8*a*-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene	compound 497, Octalox, HEOD
diethyltoluamide	*N,N*-diethyl-*m*-toluamide	
dimefox	tetramethyl phosphorodiamidic fluoride	Hanane, Pestox 14
dimetan	dimethyl 5,5-dimethyl-3-oxo-1-cyclohexen-1-yl carbamate	G-19258
dimethoate	*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate	Am. Cyanamid 12880
dimethyl carbate	dimethyl *cis*-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate	
dinitrobutylphenol	2-*sec*-butyl-4,6-dinitrophenol	Dinoseb, DNOSBP
dinitrocyclohexylphenol	2-cyclohexyl-4,6-dinitrophenol	DNOCHP, Dinex
dinitrocresol	4,6-dinitro-*o*-cresol	DNOC, DNC, Sinox
endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*-*endo*-5,8-dimethanonaphthalene	compound 269
EPN	*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate	EPN-300
ethion	*O,O,O',O'*-tetraethyl *S,S'*-methylene bisphosphorodithioate	Nialate, Niagara 1240
ethyl hexanediol	2-ethyl-1,3-hexanediol	Rutgers 612
furethrin	3-furfuryl-2-methyl-4-oxo-2-cyclopenten-1-yl chrysanthemumate	
heptachlor	3*a*,4,5,6,7,8,8-heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene	Velsicol 104, E-3314
HETP	mixture of ethyl polyphosphates containing 12 to 20% of tetraethyl pyrophosphate	hexaethyl tetraphosphate
isodrin	1,2,3,4,10,10-hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4-*endo*-*endo*-5,8-dimethanonaphthalene	compound 711
lauseto neu	chloromethyl *p*-chlorophenyl sulfone	
lindane	1,2,3,4,5,6-hexachlorocyclohexane gamma isomer of not less than 99% purity	gamma BHC
malathion	*S*-[1,2-bis(ethoxycarbonyl)ethyl] *O,O*-dimethyl phosphorodithioate	diethyl mercaptosuccinic acid, *S*-ester with *O,O*-dimethyl phosphorodithioate, malathon, compound 4049
methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane	Marlate, DMDT
methyl demeton	mixture of *O,O*-dimethyl *S*- (and *O*-)(2-ethylthio)ethyl phosphorothioates	meta-Systox

NAME TO BE USED	CHEMICAL DEFINITION	OTHER DESIGNATIONS THAT HAVE BEEN USED
methyl parathion	*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate	methyl homolog of parathion
ovex	*p*-chlorophenyl *p*-chlorobenzenesulfonate	K-6451, Ovotran
para-oxon	diethyl *p*-nitrophenyl phosphate	oxygen analog of parathion, E-600
parathion	*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate	E-605, Thiophos, compound 3422, Niran
phosphamidon	1-chloro-1-diethylcarbamoyl-1-propen-2-yl dimethyl phosphate	ML-97, OR-1191
piperonyl butoxide	*alpha*-[2-(2-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene	(butyl carbitol) (6-propylpiperonyl) ether
piperonyl cyclonene	mixture of 3-alkyl-6-carbethoxy-5-(3,4-methylenedioxyphenyl)-2-cyclohexene-1-one and 3-alkyl-5-(3,4-methylenedioxyphenyl)-2-cyclohexen-1-one	piperonyl cyclohexenone
propyl isome	dipropyl 5,6,7,8-tetrahydro-7-methylnaphtho[2,3]-1,3-dioxole-5,6-dicarboxylate	*n*-propyl isome, dipropyl 3-methyl-6,7-methylenedioxy-1,2,3,4-tetrahydronaphthalene-1,2-dicarboxylate
ronnel	*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate	Korlan, Trolene, Dow ET-57, Dow ET-14
schradan	octamethyl pyrophosphoramide	bis(dimethylamino)phosphonous anhydride, Pestox III, OMPA
sesamex	2-(2-ethoxyethoxy)ethyl 3,4-methylenedioxyphenyl acetal of acetaldehyde	Sesoxane, ENT-20871
sesamin	2,6-bis(3,4-methylenedioxyphenyl)-3-7-dioxabicyclo[3.3.0]octane	
sesamolin	2-(3,4-methylenedioxyphenyl)-6-(3,4-methylenedioxyphenoxy)-3,7-dioxabicyclo[3.3.0]octane	
siglure	*sec*-butyl 6-methyl-3-cyclohexene-1-carboxylate	ENT-21486
sulfotepp	ethyl thiopyrophosphate	*O,O,O,O*-tetraethyl-dithiopyrophosphate, dithione, Sulfatep
sulfoxide	1,2-methylenedioxy-4-[2-(octylsulfinyl)propyl] benzene	Sulfox-Cide, *n*-octyl sulfoxide of isosafrole
TDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane	DDD, Rhothane, tetrachlorodiphenylethane
TEPP	ethyl pyrophosphate, appro. 40% technical	tetraethylpyrophosphate, TEP
toxaphene	chlorinated camphene containing 67–69% of chlorine	compound 3956

OBITUARY

Harris Braley Parks
1879-1958

H. B. Parks, retired staff member of the Texas A. & M. College, passed away at his home in San Antonio on November 19. He was born June 10, 1879, at Carlinville, Illinois, and graduated with a B.S. degree from Blackburn University in 1900.

He held teaching positions in Alaska, North Carolina and Missouri prior to his appointment as Entomologist of the Texas Agricultural Extension Service in 1918. In 1922 he was appointed Apiculturist, Texas Apicultural Experiment Station, and in 1927, Chief, Division of Apiculture, in charge of a field laboratory near San Antonio. In 1945 he became Curator of the Museum and Botanist of the Tracy Herbarium, Texas A. & M. College. He retired from service in 1949.

Mr. Parks was a man of many talents, a tireless worker, a gifted observer and an inspiring teacher. He contributed articles to beekeeping, entomological and botanical journals in addition to official publications of his department. He served as Secretary of Texas Beekeepers Association, American Honey Producers League and Texas Academy of Science, was a fellow of the American Association for the Advancement of Science and was a lifelong member of the Presbyterian church.

Although Mr. Parks was in poor health for some years, his indomitable disposition and enthusiasm in his work enabled him to carry on. He was well versed in the broad as well as the more intricate aspects of zoology and botany. Among his hobbies he was especially interested in birds and other wild life and in the collection of fossil plants, shells and Indian relics. He had a wide acquaintance with collectors in various fields of interest. In company with other collectors on field trips Mr. Parks generally collected the most material and the best specimens.

He is survived by his wife Mrs. Mabel Gary Parks, one son, two daughters, one granddaughter and a sister.

ALFRED H. ALEX

BOOK REVIEW

AFRICAN IXODOIDEA. I. TICKS OF THE SUDAN (WITH SPECIAL REFERENCE TO EQUATORIA PROVINCE AND WITH REVIEWS OF THE GENERA BOOPHILUS, MARGAROPUS, AND HYALOMMA). By HARRY HOOGSTRAAL. Research Report NM 005050.29.07. [Processed]. U. S. Naval Medical Research Unit No. 3, Cairo, Egypt. 1101 pp. 372 figs., 1956.

In this outstanding work Dr. Hoogstraal has given a comprehensive view of the systematics, biology, distribution and economic importance of the tick fauna of the Sudan. It also has a broad bearing on the ticks of the entire African Continent since a high percentage of the tick species of the Continent occur in the Sudan.

Sixty-two species and two subspecies are now known from the Sudan. Of these 52 occur in Equatoria Province. The number of species and subspecies of each genus in the Sudan is as follows: *Argas* 6, *Ornithodoros* 2, *Amblyomma* 8, *Aponomma* 2, *Boophilus* 2, *Dermacentor* 2, *Haemaphysalis* 8, *Ixodes* 7, *Margaropus* 1, *Rhipicephalus* 19.

Each species is treated under the headings—"Distribution in the Sudan," "Distribution," "Hosts," "Biology," "Disease Relations," "Remarks" and "Identification."

The distribution of all species by Province is presented in tabular form. Under "Distribution in the Sudan" all known collection-localities are listed for each species by Province with more detailed information as to stages, hosts, and dates of collection for the records in Equatoria. Under "Distribution" the occurrence of each species in the Countries of Africa is given with literature citations. Likewise host records are given in detail, frequently grouped by parasitic stage on each host species. Biological data are given in some detail especially in the case of economically important species. Biological information is cited or abstracted whether obtained in Africa or elsewhere. Ecological information is frequently stressed under a subheading. "Disease Relations" are summarized very briefly under hosts affected. Although the author explains that this field will be adequately covered in a subsequent volume, it would have added materially if citations to the more important articles on this subject had been included.

During the 8 years Dr. Hoogstraal has been assigned to work on the ticks of the Sudan, he has not only done a splendid job of reviewing the entire subject but he has added an immense amount of new information. The economically important species are considered at length. This is particularly true of *Argas persicus* Oken, the fowl tick, which occurs widely in the southwestern U. S., pp. 59–74; the notorious carrier of African tick-borne relapsing fever—*Ornithodoros moubata* Murray, pp. 220–290; the brown ear tick, *Rhipicephalus appendiculatus* Neum., an important vector of East Coast fever of Africa, pp. 600–615; another carrier of East Coast fever and other diseases, *R. evertsi evertsi* Neum., the red tick, pp. 642–653; and the bane of dog owners in the U. S., *R. sanguineus* Latr. pp. 686–724. The glossy tick, *R. simus simus* Koch, although not known to be of particular economic importance also received rather full treatment, pp. 730–759.

The author concludes there are only three species of *Boophilus* in Africa; one is the Texas fever tick, *B. annulatus* Say, which is now eradicated from the United States, another *B. microplus* Canestrini which occurs in Mexico and southward and which occasionally crosses into Texas along the Rio Grande. This species was also found in Florida but has been eradicated. The third is *B. decoloratus* Koch, the well known "blue tick" of Africa, transmitter of red water of cattle and other diseases. Although *B. annulatus* occurs in West Africa, central Africa, and in certain parts of southern Sudan, it is not regarded as especially important. It has not been studied biologically in Africa. The author regards *B. congolensis* Minning and *B. calcaratus* Birula as synonyms of *B. annulatus*. In Africa this species is almost exclusively a parasite of cattle.

The large number of species of *Rhipicephalus* and the economic importance of so many forms of that genus in Africa bear out the statement that Continental Africa is the place of origin and the center of distribution of species of that genus. Hoogstraal points out that of the 46 species and subspecies of the genus recognized by Zumpt in his preliminary revision of the genus 39 are endemic in the Ethiopian Faunal Region.

Keys to families, genera and species are included. These relate to males and females—not to the immature stages. These keys are extremely useful to all workers dealing with ticks. Perhaps the author has placed too much dependence on the female genitalia as a character. This feeling may be due to the fact that this character has come into use very recently as a result of taxonomic studies by Dr. Feldman-Muhsam.

Dr. Hoogstraal frequently points out changes in the appearance of structures due to degree of engorgement of specimens. He also includes helpful information on the wide limits of variation in some species.

Although under the heading "Identification" excellent diagnostic features of each species are presented, workers not familiar with African ticks will wish for more complete descriptions. Obviously in a publication of this type it would be impractical to include full descriptions. The excellent drawings of the dorsal and ventral sides of both males and females are most helpful in making identifications, however the method of reproducing these drawings obscures important details especially in very dark colored species. Outline drawings of diagnostic characters, as given for *Margaropus*, would have been a great help to toxonomists. The author has made a useful contribution by pointing out distinguishing characters in the palpi and first coxae of females of *B. annulatus* and *B. microplus*.

One new species, *Margaropus reidi* is fully described in this work.

Common names are given for each species discussed. This is commendable as it will tend to get appropriate names fixed even though they are not in use today. It is noted that, unfortunately, in some instances common names accepted by the Entomological Society of America are not used.

The author's suggestions for work needed should be helpful to workers concerned with species occurring in Africa. Of special usefulness is the author's summarization of publications in several languages by the many authors who have dealt with ticks found in the Sudan. It is interesting to note that through Dr. Hoogstraals's energy and the cooperation of other workers, literature covering the entire field has been assembled in his own library. The bibliography he has included in this volume contains 1880 titles, of these 20 are by the author himself and three by him as senior author with others.

Climatic and biotic features of the Sudan are treated briefly but adequately for the purpose. Numerous charts give information on mean temperatures, mean rainfall by months, floral districts and faunal areas based on Tabanidae and on tick distribution in the Sudan. In the latter nine faunal areas are mapped and the tick fauna discussed.

In the appendix of 49 pages the author has presented, by species, additional data and references obtained after the main body of the text was prepared. Notes and drawings are also included on several forms some of which were not given definite specific rank. Of particular value are drawings of the tarsi, palpi, hypostomes and spiracular plates of male, female and nymph *Margaropus reidi* n. sp. and of male and female *M. winthemi* Karsch.

We trust that the Medical Department of the U. S. Navy, which has made this work possible, will be in position to supply every worker on ticks and tick problems throughout the world with a copy of this valuable volume and of those in preparation.

The foreword is written by the eminent Medical Entomologist, Dr. C. B. Philip, and the Volume is dedicated to an outstanding early investigator of American ticks, with these words: "To Colonel Willard Van Orsdel King, Ph.D., a veteran Medical Entomologist and pioneer worker on tick vectors of Rocky Mountain spotted fever, from whom I have learned much of the methods of scientific inquiry."

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